(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification⁷: G01N 33/574

(21) International Application Number: PCT/US01/44782

(22) International Filing Date:
27 November 2001 (27.11.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/253,361	27 November 2000 (27.11.2000)	US
60/255,370	13 December 2000 (13.12.2000)	US
60/256,027	15 December 2000 (15.12.2000)	US
60/258,157	22 December 2000 (22.12.2000)	US
60/259,615	3 January 2001 (03.01.2001)	US
60/260,186	5 January 2001 (05.01.2001)	US
60/266,169	2 February 2001 (02.02.2001)	US
60/266,929	6 February 2001 (06.02.2001)	US
60/278,093	23 March 2001 (23.03.2001)	US
60/289,444	7 May 2001 (07.05.2001)	US
60/294,887	31 May 2001 (31.05.2001)	US
60/298,272	14 June 2001 (14.06.2001)	US

(71) Applicant: **MINERVA BIOTECHNOLOGIES CORPORATION** [US/US]; 142 Church Street, Newton, MA 02458 (US).

(72) Inventors: **BAMDAD, Cynthia, C.**; 142 Church Street, Newton, MA 02458 (US). **BAMDAD, R., Shoshana**; 142 Church Street, Newton, MA 02458 (US).

(74) Agent: **POMIANEK, Michael, J.**; Wolf, Greenfield & Sacks, P.C., 600 Atlantic Avenue, Boston, MA 02210 (US).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM). European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: DIAGNOSTIC TUMOR MARKERS, DRUG SCREENING FOR TUMORIGENESIS INHIBITION, AND COMPOSITIONS AND METHODS FOR TREATMENT OF CANCER

(57) Abstract: The invention provides a series of compositions, methods, kits, articles and species associated primarily with the diagnosis and/or treatment of cell proliferation, specifically cancer. Cell proliferation associated with aberrant expression of MUCI is particularly focused upon. Mechanisms associated with MUCI cell proliferation are discussed.

DIAGNOSTIC TUMOR MARKERS, DRUG SCREENING FOR TUMORIGENESIS INHIBITION, AND COMPOSITIONS AND METHODS FOR TREATMENT OF CANCER

5 **Related Applications**

This non-provisional application claims the benefit under Title 35, U.S.C. §119(e) of co-pending U.S. provisional application no. 60/253,361, filed November 27, 2000, U.S. provisional application no. 60/255,370, filed December 13, 2000, U.S. provisional application no. 60/256,027, filed December 15, 2000, U.S. provisional

10 application no. 60/258,157, filed December 22, 2000, U.S. provisional application no. 60/259,615, filed January 03, 2001, U.S. provisional application no. 60/260,186, filed January 05, 2001, U.S. provisional application no. 60/266,169, filed February 02, 2001, U.S. provisional application no. 60/289,444, filed May 07, 2001, U.S. provisional application no. 60/266,929, filed February 06, 2001, U.S. provisional application no.

15 60/278,093, filed March 23, 2001, U.S. provisional application no. 60/294,887, filed May 31, 2001, and U.S. provisional application no. 60/298,272, filed June 14, 2001, each of which is incorporated by reference herein.

Field of the Invention

20 The invention relates to assays using shed cell surface receptor interchain binding regions and cleavage products for cancer diagnosis, and for the evaluation of cancer treatment and using the portion of the receptor that remains on the cell as a molecular target for cancer therapeutics.

25 **Background of the Invention**

Many of the biomolecular interactions that promote tumorigenesis involve cell surface proteins that mediate both intra- and intercellular signaling. "Tumor markers" are proteins on the surface of a cell that are exclusively expressed, over-expressed or show an altered expression pattern as a result of transformation to a neoplastic state. The

30 surface concentration of certain tumor markers has been correlated to the progression of cancer. For example, the interaction between the cell surface receptor $\alpha V\beta 3$ and the cell adhesion molecule vitronectin has been implicated in angiogenesis (Varner J, Cheresh D: Integrins and cancer. *Curr Opin Cell Biol*, 1996, 8(5): 724-730; Vailhe B, Ronot X, Tracqui P, Usson Y, Tracqui L: In vitro angiogenesis is modulated by the mechanical

properties of fibrin gels and is related to αVβ3 integrin localization. *In Vitro Cell Dev Biol Anim*, 1997, **33**(10): 763-773; Horton M: The aVb3 integrin "vitronectin receptor". *Int J Biochem Cell Biol*, 1997, **29**(5): 721-725) and the increased concentration of αVβ3 on melanoma cells has been correlated with poor prognosis (Hieken T, Farolan M,

5   Ronan S, Shilkaitis A, Wild L, Das Gupta T: β3 integrin expression in melanoma predicts subsequent metastasis. *J Surg Res*, 1996, **63**(1): 169-173).

Cell surface receptors, that have been linked to cancer, make up an important class of therapeutic targets. Many pharmaceutical companies are actively involved in screening drug libraries for compounds that bind to and block these cell surface

10   receptors. For example, an important drug used to treat breast cancer is Herceptin (Pegram M, Lipton A, Hayes D, Webber B, Baselga J, Tripathy D, Baly D, Baughman S, Twaddell T, Glaspy J, Slamon D: Phase II study of receptor-enhanced chemosensitivity using recombinant humanized anti-p185 Her2/neu monoclonal antibody plus cisplatin, in patients with Her2/neu-overexpressing metastatic breast cancer refractory to

15   chemotherapy treatment, *J Clin Oncol*, 1998, **16**(8): 2659-2671). This drug binds to and blocks HER2/neu (Ross J, Fletcher J: review, The Her2/neu oncogene in breast cancer: prognostic factor, predictive factor, and target for therapy. *Stem Cells*, 1998, **16**(6): 413-428) which is a cell surface receptor that is over-expressed on 30% of breast tumors.

Another cell surface receptor, called MUC1 (Treon S, Mollick J, Urashima M,

20   Teoh G, Chauhan D, Ogata A, Raje N, Hilgers J, Nadler L, Belch A, Pilarski L and Anderson K: MUC1 core protein is expressed on multiple myeloma cells and is induced by dexamethasone. *Blood*, 1999, **93**(4): 1287-1298), is especially interesting since it is aberrantly expressed on many human tumors, including 80% of breast tumors, and on a significant percentage of prostate, lung, ovarian, colorectal and perhaps brain, cancers.

25   On healthy secretory epithelium, MUC1 is clustered at the apical border and is not expressed over other portions of the cell. However, in tumor cells, the receptor is homogeneously over-expressed over the entire cell surface (Kufe D., Inghirami G., Abe M., Hayes D, Justi-Wheeler H, Schlom J: Differential reactivity of a novel monoclonal antibody (DF3) with human malignant versus benign breast tumors. *Hybridoma*, 1984, **3**:

30   223-232), rather than just at the apical border. It is also known that women with breast cancer have elevated levels of shed MUC1 receptor in their blood stream. Extracellular portions of the MUC1 receptor are cleaved or "shed", by at least one enzyme, and

released into the blood stream. Levels of shed MUC1 receptor in serum are measured to track breast cancer patients for recurrence. However, the method is too variable and insensitive to be used as a general diagnostic.

Until now, the mechanistic link between the MUC1 receptor and tumorigenesis has not been understood. Attempts to correlate the number of repeat units, which varies from person to person, and susceptibility to cancer failed. Investigations of a possible connection, between glycosylation of the MUC1 receptor and cancer, produced conflicting results. Importantly, until now, a functional ligand(s) for the extracellular portion of the MUC1 receptor has not been identified.

Absent an understanding of the mechanism of the MUC1 receptor, and how it triggers tumorigenesis, it has not been possible to design or identify therapeutics that interfere with the disease-associated function of this receptor. Indeed, currently there is no drug in use or, to our knowledge, in clinical trials that is known to target the MUC1 receptor.

The present invention describes discoveries that elucidate critical aspects of the mechanism by which MUC1 triggers cell proliferation and tumorigenesis. These discoveries provide novel molecular targets for drug screening assays which the inventors have used to identify compounds that inhibit the MUC1-dependent tumorigenesis. These discoveries also enable an early diagnostic assay.

Summary of the Invention

The present invention provides a variety of kits, methods, compositions, peptide species and articles associated with cell proliferation, specifically cancer. The invention involves primarily techniques and components for the diagnosis and treatment of cancer.

In one aspect, the invention provides a series of kits. One kit includes a first article having a surface, and a peptide sequence immobilized relative to or adapted to be immobilized relative to the surface. The peptide sequence includes a portion of a cell surface receptor that interacts with an activating ligand such as a growth factor to promote cell proliferation. Also included in the kit is a candidate drug for affecting the ability of the peptide sequence to bind to other identical peptide sequences in the presence of the activating ligand. The portion includes enough of the cell surface receptor to interact with the activating ligand and the portion is free of interchain binding region to the extent necessary to prevent spontaneous binding between the portions.

Another kit of the invention comprises a species able to become immobilized relative to a shed cell surface receptor interchain binding region, and a signaling entity immobilized relative to or adapted to be immobilized relative to the species.

Another kit of the invention comprises a species able to bind to a portion of a cell

5   surface receptor that remains attached to the cell surface after shedding of a cell surface· receptor interchain binding region, and a signaling entity immobilized relative to or adapted to be immobilized relative to the species.

Another kit of the invention comprises a species able to bind to a portion of a cell surface receptor that includes the interchain binding region, and a signaling entity

10   immobilized relative to or adapted to be immobilized relative to the species.

Another kit of the invention comprises an article (which can be a particle), and at least a fragment of the sequence that corresponds to that portion of a cell surface receptor that interacts with an activating ligand such as a growth factor to promote cell proliferation, the fragment being detached from any cell, fastened to or adapted to be

15   fastened to the article.

Another kit of the invention comprises an article having a surface, and a biomolecule that binds to a portion of a cell surface receptor that interacts with an activating ligand such as a growth factor to promote cell proliferation. The biomolecule is fastened to or adapted to be fastened to the surface of the article.

20   In another aspect, the invention provides a series of methods. One method comprises providing a peptide including a portion of a cell surface receptor that interacts with an activating ligand such as a growth factor to promote cell proliferation, exposing the peptide to a candidate drug for affecting the ability of the activating ligand to interact with the peptide, and to the activating ligand, and determining the ability of the candidate

25   drug to prevent interaction of the activating ligand with the peptide. The portion includes enough of the cell surface receptor to interact with the activating ligand and the portion is free of interchain binding region to the extent necessary to prevent spontaneous binding between portions.

Another method of the invention involves treating a subject having cancer or

30   being at risk for developing cancer, the method comprises administering to the subject an agent that reduces cleavage of a cell surface receptor.

Another method of the invention for treating a subject having cancer or at risk for developing cancer comprises administering to the subject an agent that reduces cleavage of a cell surface receptor interchain binding region from the cell surface.

Another method of the invention comprises determining an amount of cleavage
5   of a cell surface receptor interchain binding region from a cell surface, and evaluating indication of cancer or potential for cancer based upon the determining step.

Another method of the invention comprises determining a site of cleavage of a cell surface receptor in a sample from a subject, and evaluating an indication of cancer or potential for cancer based upon the determining step.

10 Another method of the invention involves determining a cleavage site of a cell surface. The method comprises contacting a cell with an agent that binds specifically to one potential cell surface receptor cleavage site and another agent that binds specifically to another potential cell surface receptor cleavage site. The ratio of binding of the two agents to the cell surface is compared in the method.

15 Another method of the invention comprises determining a first amount of cleavage of a cell surface receptor interchain binding region from a cell surface of a sample from a subject. A second amount of cleavage of cell surface receptor interchain binding region from a cell surface of a sample from the subject is also determined, and the first amount is compared to the second amount.

20 Another method of the invention involves treating a subject to reduce the risk of or progression of cancer. The method comprises administering to a subject, who is known to be at risk for cancer or is diagnosed with cancer, an agent for inhibiting interaction of an activating ligand with a portion of a cell surface receptor that interacts with the activating ligand to promote cell proliferation.

25 Another method of the invention involves treating a subject to reduce the risk of or progression of cancer. The method comprises administering to a subject, who is known to be at risk of cancer or is diagnosed with cancer, an agent for preventing clustering of portions of cell surface receptors that interact with an activating ligand such as a growth factor to promote cell proliferation.

30 Another method of the invention comprises exposing a ligand capable of binding with a portion of a cell surface receptor that remains attached to the cell after shedding of the cell surface receptor interchain binding region, and an agent capable of blocking this

binding, to a candidate drug for disruption of interaction between the ligand and the agent. Disruption of the interaction by the candidate drug is determined.

Another method of the invention comprises exposing a portion of a cell surface receptor that remains attached to the cell surface after shedding of a cell surface receptor interchain binding region which is capable of binding with a ligand, and an agent capable of blocking this binding, to a candidate drug for disruption of interaction between the portion and the agent, and determining disruption of the interaction by the candidate drug.

Another method of the invention comprises exposing a synthetic drug, and a biological target of the synthetic drug, to a candidate drug which may interact with a biological target to a degree greater than the interaction between the synthetic drug and the target, and determining disruption of the interaction by the candidate drug.

Another method involves diagnosing a physiological state indicative of cancer or potential for cancer. The method comprises determining a specific cleavage site of MUC1 distinguishable from a different cleavage state of MUC1.

Another method of the invention involves treating a subject having a cancer characterized by the aberrant expression of MUC1, comprising administering to the subject etomoxir in an amount effective to reduce tumor growth.

Another method of the invention involves treating a subject having a cancer characterized by the aberrant expression of MUC1, comprising administering to the subject L-α-methyl-dopa in an amount effective to reduce tumor growth.

Another method of the invention for treating a subject having cancer characterized by the aberrant expression of MUC1, comprises administering to the subject calcimycin in an amount effective to reduce tumor growth.

Another method for treating a subject having a cancer characterized by the aberrant expression of MUC1, comprises administering to the subject butylindazole in an amount effective to reduce tumor growth.

In another aspect, the invention provides compositions. One composition of the invention comprises at least a portion of a shed cell surface receptor interchain binding region, and a signaling entity immobilized relative to or adapted to be immobilized relative to the portion.

The invention also provides peptide species. One peptide species of the invention comprises at least a fragment of a sequence that corresponds to that portion of a cell surface receptor that interacts with an activating ligand such as a growth factor to promote cell proliferation, the portion being detached from any cell, and an affinity tag.

Brief Description of the Drawings

Fig. 1 is a schematic illustration of the MUC1 receptor;

Fig. 2 is a black and white photocopy of an image from a colloid-based, color change binding experiment that shows which portions of soluble MUC1 bind to each other or self-aggregate;

Fig. 3 is a black and white photocopy of an image from a colloid-based color change experiment in which the ability of peptides to self-aggregate was used to help determine a boundary between a portion of the MUC1 receptor that self-aggregates the cell-proximal portion that does not; results imply a disease-related cleavage site on the MUC1 receptor;

Fig. 4 is a graph of percent cell proliferation that shows that an antibody against an epitope of the MUC1 receptor which is proximal to the cell surface, and that dimerizes the receptor, enhances cell proliferation in a manner typical of a growth factor/receptor – antibody interaction;

Fig. 5 is a graph of percent cell proliferation that shows that an antibody against an epitope of the MUC1 receptor which is proximal to the cell surface, and that dimerizes the receptor, dramatically enhances cell proliferation;

Fig. 6 is a black and white photocopy of an image of a section of a 96-well plate illustrating a color change assay in which a ligand(s) present in the lysates of cells that express MUC1, binds to and dimerize the His-PSMGFR peptide, derived from MUC1, which is immobilized on gold colloids, while lysates from cells that do not express MUC1 do not;

Fig. 7 is a black and white photocopy of an image of 96-well plate illustrating a colloid-based color-change binding assay between a MUC1-derived peptide and a ligand(s) present in a crude cell lysate; addition of imidazole, which releases the probe peptide from the colloid, causes a reversal of the color change, which argues that the color change is the result of a specific interaction rather than random colloid aggregation;

Figs. 8A-D is a black and white photocopy of an image that shows a colloid-based color change assay in 96-well plates in which a ligand present in a cell lysate caused dimerization of a MUC1-derived peptide and that the degree of color change, which indicates an amount of ligand present, was a function of which cell line supplied the lysate;

5

Fig. 9 is a black and white photocopy of a silver-stained gel showing ligands that were fished out of cell lysates using the PSMGFR peptide, in the presence of the protease inhibitor PMSF;

Fig. 10 is a black and white photocopy of a silver-stained gel showing ligands that were

10 fished out of cell lysates using the PSMGFR peptide, in the absence of the protease inhibitor PMSF;

Fig. 11 is a black and white photocopy of an image of a 96-well plate illustrating a color-change binding assay between a MUC1-derived peptide and a ligand(s) present in a crude cell lysate from cells that overexpress MUC1;

15 **Fig. 12** is a black and white photocopy of an image of a 96-well plate illustrating a color-change drug-screening assay used to detect inhibitors of the MUC1-Ligand interaction;

Fig. 13 shows a histogram illustrating the selective inhibition of proliferation of tumor cells that aberrantly express the MUC1 receptor, in response to treatment with

20 compounds of the invention, and lack of an effect on cells that do not express MUC1;

Fig. 14 is a black and white photocopy of an image of a 96-well plate illustrating a color-change drug-screening assay identifying several compounds that intefere with the interaction of the MGFR portion of the MUC1 receptor and a multimerizing ligand(s);

Fig. 15 shows a histogram illustrating the selective inhibition of proliferation of tumor

25 cells that aberrantly express the MUC1 receptor, in response to treatment with drugs that specifically inhibit MUC1 positive cells;

Fig. 16 shows a histogram illustrating the nonselective inhibition of proliferation of cells in response to treatment with drugs that non-specifically inhibit cell proliferation;

Fig. 17 shows a histogram illustrating that drugs that selectivly inhibit proliferation of

30 tumor cells that aberrantly express the MUC1 receptor bind to the PSMGFR, while drugs that non-selectively inhibit cell proliferation do not;

Fig. 18 is a graph showing that the inhibition of MUC1-dependent cell proliferation induced by an anti-tumor drug identified in accordance with the invention, is modulated

when a synthetic peptide, corresponding to the portion of MUC1 that remains at the cell

following cleavage, competitively inhibits the drug-cell surface receptor interaction;

Fig. 19 is a black and white photocopy of a comassie blue-stained gel showing that the

PSMGFR peptide runs at an apparently higher molecular weight after incubation with

5   cell;

Fig. 20 is a black and white photocopy of an image of a 96-well plate illustrating a

color-change ligand binding assay illustrating that inhibiting enzymatic modification of

PSMGFR prevents it binding to ligands.

10   **Detailed Description of the Invention**

Definitions:

The term "MUC1 Growth Factor Receptor" (MGFR) is a functional definition

meaning that portion of the MUC1 receptor that interacts with an activating ligand, such

as a growth factor, to promote cell proliferation. The MGFR region of MUC1 is that

15   portion that is closest to the cell surface and is defined by most or all of the PSMGFR.

The MGFR is inclusive of both unmodified peptides and peptides that have undergone

enzyme modifications, such as, for example, phosphorylation, glycosylation, etc.

Results of the invention are consistent with a mechanism in which this portion is made

accessible to the ligand upon MUC1 cleavage at a site associated with tumorigenesis that

20   causes release of the IBR from the cell.

The term "Interchain Binding Region" (IBR) is a functional definition meaning

that portion of the MUC1 receptor that binds strongly to identical regions of other MUC

1 molecules giving MUC1 the ability to aggregate (i.e. self-aggregate) with other MUC1

receptors via the IBRs of the respective receptors. This self-aggregation may contribute

25   to MUC1 receptor clustering, observed in healthy cells.

In a preferred embodiment, the IBR may be approximately defined as a stretch of

at least 12 to 18 amino acid sequence within the region of the human MUC1 receptor

defined as comprising amino acids 507 to 549 of the extracellular sequence of the MUC1

receptor, with amino acids 525 through 540 and 525 through 549 especially preferred

30   (numbers refer to Andrew Spicer *et al.*, J. Biol. Chem Vol 266 No. 23, 1991 pgs. 15099-

15109; these amino acid numbers correspond to numbers 1067, 1109, 1085, 1100, 1085,

1109 of Genbank accession number P15941; PID G547937, SEQ ID NO: 10) or fragments, functional variants or conservative substitutions thereof.

The term "cleaved IBR" means the IBR (or a portion thereof) that has been released from the receptor molecule segment which remains attached to the cell surface. The release may be due to enzymatic or other cleavage of the IBR. As used herein, when the IBR is "at the surface of a cell", it means the IBR is attached to the portion of the cell surface receptor that has not been shed, or cleaved. The cleaved IBR of interest is a "disease-associated cleavage", i.e. that type of cleavage that can result in cancer.

The term "Constant Region" (CR) is any non-repeating sequence of MUC1 that exists in a 1:1 ratio with the IBR and forms part of the portion of MUC1 that is shed upon cleavage in healthy and tumorigenesic cells.

The term "Repeats" is given its normal meaning in the art.

The term "Primary Sequence of the MUC1 Growth Factor Receptor" (PSMGFR) is a peptide squence, defined below (See Table 1 – SEQ ID NO: 7), that defines most or all of the MGFR. The PSMGFR is inclusive of both unmodified peptides and peptides that have undergone enzyme modifications, such as, for example, phosphorylation, glycosylation, etc. The histidine-tagged PSMGFR (See Table 1 – SEQ ID NO: 2) is abbreviated herein as His-PSMGFR.

The term "Extended Sequence of the MUC1 Growth Factor Receptor" (ESMGFR) is a peptide squence, defined below (See Table 1 – SEQ ID NO: 3), that defines all of His-PSMGFR plus 9 amino acids of the proximal end of PSIBR.

PSIBR is a peptide squence, defined below (See Table 1 – SEQ ID NO: 8), that defines most or all of the IBR.

The term "separation" means physical separation from a cell, i.e. a situation in which a portion of MUC 1 that was immobilized with respect to a cell is no longer immobilized with respect to that cell. E.g. in the case of cleavage of a portion of MUC 1, the portion that is cleaved is "separated" if it is free to migrate away from the cell and thereafter may be detected in a bodily fluid, or immobilized at a location remote from the cell from which it was cleaved such as another cell, a lymph node, etc.

The term "binding" refers to the interaction between a corresponding pair of molecules that exhibit mutual affinity or binding capacity, typically specific or non-specific binding or interaction, including biochemical, physiological, and/or pharmaceutical interactions. Biological binding defines a type of interaction that occurs

between pairs of molecules including proteins, nucleic acids, glycoproteins, carbohydrates, hormones and the like. Specific examples include antibody/antigen, antibody/hapten, enzyme/substrate, enzyme/inhibitor, enzyme/cofactor, binding protein/substrate, carrier protein/substrate, lectin/carbohydrate, receptor/hormone,

5   receptor/effector, complementary strands of nucleic acid, protein/nucleic acid repressor/inducer, ligand/cell surface receptor, virus/ligand, etc.

The term "binding partner" refers to a molecule that can undergo binding with a particular molecule. Biological binding partners are examples. For example, Protein A is a binding partner of the biological molecule IgG, and vice versa.

10   The term "aggregate" (noun) means a plurality of cell surface receptors or fragments thereof (e.g. MUC 1) immobilized with respect to each other with or without an intermediate auxialliary to the host system. This includes self-aggregation of healthy receptors at a cell surface; self-aggregation of cleaved receptors or fragments bound to each other; cleaved receptors or fragments bound to receptors or fragments attached to a

15   cell surface; receptors or fragments, whether attached to a cell or cleaved, immobilized with respect to each other via an intermediate auxialliary to the host. "Intermediate auxialliary to the host system" includes a synthetic species such as a polymer, dendrimer, etc., or a naturally-occurring species, for example an IgM antibody, which is not simply naturally present in the host system but is added to the host system from a source

20   external to the host system. This excludes aggregation that is the result of an intermediate naturally present in the host system such as a growth factor that can cause disease-associated aggregation ("Inductive multimerization"). "Aggregate" (verb) or "aggregation" means the process of forming an aggregate (noun).

"Inductive multimerization" refers to aggregation wherein the aggregate formed

25   can act to induce the cells to grow or proliferate. Inductive multimerization typically involves dimerization or tetramerization of cell surface receptors, for example by a growth factor or other activating ligand, but can also involve higher order multimerization, so long as the degree of multimerization is not so great as to mimic natural receptor clustering, in a particular cell type, which prevents receptors from

30   signalling the cell to grow or proliferate.

"Preventative clustering" refers to multimerization of receptors to form an aggregate involving a sufficient number of receptors to mimic natural receptor clustering,

in a particular cell type, which prevents receptors from signalling the cell to grow or proliferate, for example with an intermediate auxialliary to the host system.

A "ligand" to a cell surface receptor, refers to any substance that can interact with the receptor to temporarily or permanantly alter its structure and/or function. Examples

5   include, but are not limited to binding partners of the receptor and agents able to alter the chemical structure of the receptor (e.g. modifying enzymes).

An "activating ligand" refers to a ligand able to effect inductive multimerization of cell surface receptors. Activating ligands can include, but are not limited to, a single molecular species with greater than one active site able to bind to a receptor; a dimer, a

10   tetramer, a higher multimer, or a complex comprising a plurality of molecular species. In the context of MUC1 tumor cells, an activating ligand can be a species produced by the cells that interacts with the MGFRs on the surface of the MUC1 tumor cells in a manner that effects inductive multimerization.

A "growth factor" refers to a species that may or may not fall into a class of

15   previously-identified growth factors, but which acts as a growth factor in that it acts as an activating ligand.

A "MUC1 presenting cell" refers to both non-cancerous and cancerous cells expressing MUC1 and/or MGFRs on the surface. A "MUC1 tumor cell" or "MUC1 cancer cell" or "cancerous MUC1 cell" refers to a cancerous tumor cell that aberrantly

20   expresses MUC1 and/or MGFR on its surface.

"Colloids", as used herein, means nanoparticles, i.e. very small, self-suspendable or fluid-suspendable particles including those made of material that is, e.g., inorganic or organic, polymeric, ceramic, semiconductor, metallic (e.g. gold), non-metallic, crystalline, amorphous, or a combination. Typically, colloid particles used in accordance

25   with the invention are of less than 250 nm cross section in any dimension, more typically less than 100 nm cross section in any dimension, and in most cases are of about 2-30 nm cross section. One class of colloids suitable for use in the invention is 10-30 nm in cross section, and another about 2-10 nm in cross section. As used herein this term includes the definition commonly used in the field of biochemistry.

30   As used herein, a component that is "immobilized relative to" another component either is fastened to the other component or is indirectly fastened to the other component, e.g., by being fastened to a third component to which the other component also is fastened, or otherwise is transitionally associated with the other component. For

example, a signaling entity is immobilized with respect to a binding species if the signaling entity is fastened to the binding species, is fastened to a colloid particle to which the binding species is fastened, is fastened to a dendrimer or polymer to which the binding species is fastened, etc. A colloid particle is immobilized relative to another

5   colloid particle if a species fastened to the surface of the first colloid particle attaches to an entity, and a species on the surface of the second colloid particle attaches to the same entity, where the entity can be a single entity, a complex entity of multiple species, a cell, another particle, etc.

"Signaling entity" means an entity that is capable of indicating its existence in a

10   particular sample or at a particular location. Signaling entities of the invention can be those that are identifiable by the unaided human eye, those that may be invisible in isolation but may be detectable by the unaided human eye if in sufficient quantity (e.g., colloid particles), entities that absorb or emit electromagnetic radiation at a level or within a wavelength range such that they can be readily detected visibly (unaided or with

15   a microscope including an electron microscope or the like), or spectroscopically, entities that can be detected electronically or electrochemically, such as redox-active molecules exhibiting a characteristic oxidation/reduction pattern upon exposure to appropriate activation energy ("electronic signaling entities"), or the like. Examples include dyes, pigments, electroactive molecules such as redox-active molecules, fluorescent moieties

20   (including, by definition, phosphorescent moieties), up-regulating phosphors, chemiluminescent entities, electrochemiluminescent entities, or enzyme-linked signaling moieties including horseradish peroxidase and alkaline phosphatase. "Precursors of signaling entities" are entities that by themselves may not have signaling capability but, upon chemical, electrochemical, electrical, magnetic, or physical interaction with another

25   species, become signaling entities. An example includes a chromophore having the ability to emit radiation within a particular, detectable wavelength only upon chemical interaction with another molecule. Precursors of signaling entities are distinguishable from, but are included within the definition of, "signaling entities" as used herein.

As used herein, "fastened to or adapted to be fastened", in the context of a species

30   relative to another species or to a surface of an article, means that the species is chemically or biochemically linked via covalent attachment, attachment via specific biological binding (e.g., biotin/streptavidin), coordinative bonding such as chelate/metal binding, or the like. For example, "fastened" in this context includes multiple chemical

linkages, multiple chemical/biological linkages, etc., including, but not limited to, a
binding species such as a peptide synthesized on a polystyrene bead, a binding species
specifically biologically coupled to an antibody which is bound to a protein such as
protein A, which is attached to a bead, a binding species that forms a part (via genetic
engineering) of a molecule such as GST or Phage, which in turn is specifically
biologically bound to a binding partner covalently fastened to a surface (e.g., glutathione
in the case of GST), etc. As another example, a moiety covalently linked to a thiol is
adapted to be fastened to a gold surface since thiols bind gold covalently. Similarly, a
species carrying a metal binding tag is adapted to be fastened to a surface that carries a
molecule covalently attached to the surface (such as thiol/gold binding) which molecule
also presents a chelate coordinating a metal. A species also is adapted to be fastened to a
surface if a surface carries a particular nucleotide sequence, and the species includes a
complementary nucleotide sequence.

"Covalently fastened" means fastened via nothing other than one or more
covalent bonds. E.g. a species that is covalently coupled, via EDC/NHS chemistry, to a
carboxylate-presenting alkyl thiol which is in turn fastened to a gold surface, is
covalently fastened to that surface.

"Specifically fastened" or "adapted to be specifically fastened" means a species is
chemically or biochemically linked to another specimen or to a surface as described
above with respect to the definition of "fastened to or adapted to be fastened", but
excluding all non-specific binding.

Certain embodiments of the invention make use of self-assembled monolayers
(SAMs) on surfaces, such as surfaces of colloid particles, and articles such as colloid
particles having surfaces coated with SAMs. In one set of preferred embodiments,
SAMs formed completely of synthetic molecules completely cover a surface or a region
of a surface, e.g. completely cover the surface of a colloid particle. "Synthetic
molecule", in this context, means a molecule that is not naturally occurring, rather, one
synthesized under the direction of human or human-created or human-directed control.
"Completely cover" in this context, means that there is no portion of the surface or
region that directly contacts a protein, antibody, or other species that prevents complete,
direct coverage with the SAM. I.e. in preferred embodiments the surface or region
includes, across its entirety, a SAM consisting completely of non-naturally-occurring
molecules (i.e. synthetic molecules). The SAM can be made up completely of SAM-

forming species that form close-packed SAMs at surfaces, or these species in combination with molecular wires or other species able to promote electronic communication through the SAM (including defect-promoting species able to participate in a SAM), or other species able to participate in a SAM, and any combination of these.

5   Preferably, all of the species that participate in the SAM include a functionality that binds, optionally covalently, to the surface, such as a thiol which will bind to a gold surface covalently. A self-assembled monolayer on a surface, in accordance with the invention, can be comprised of a mixture of species (e.g. thiol species when gold is the surface) that can present (expose) essentially any chemical or biological functionality.

10   For example, they can include tri-ethylene glycol-terminated species (e.g. tri-ethylene glycol-terminated thiols) to resist non-specific adsorption, and other species (e.g. thiols) terminating in a binding partner of an affinity tag, e.g. terminating in a chelate that can coordinate a metal such as nitrilotriacetic acid which, when in complex with nickel atoms, captures a metal binding tagged-species such as a histidine-tagged binding

15   species. The present invention provides a method for rigorously controlling the concentration of essentially any chemical or biological species presented on a colloid surface or any other surface. Without this rigorous control over peptide density on each colloid particle, co-immobilized peptides would readily aggregate with each other to form micro-hydrophobic-domains that would catalyze colloid-colloid aggregation in the

20   absence of aggregate-forming species present in a sample. This is an advantage of the present invention, over existing colloid agglutination assays. In many embodiments of the invention the self-assembled monolayer is formed on gold colloid particles.

The kits described herein, contain one or more containers, which can contain compounds such as the species, signaling entities, biomolecules, and/or particles as

25   described. The kits also may contain instructions for mixing, diluting, and/or administrating the compounds. The kits also can include other containers with one or more solvents, surfactants, preservative and/or diluents (e.g. normal saline (0.9% NaCl, or 5% dextrose) as well as containers for mixing, diluting or administering the components to the sample or to the patient in need of such treatment.

30   The compounds in the kit may be provided as liquid solutions or as dried powders. When the compound provided is a dry powder, the powder may be reconstituted by the addition of a suitable solvent, which also may be provided. Liquid forms of the compounds may be concentrated or ready to use. The solvent will depend

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on the compound and the mode of use or administration. Suitable solvents for are well known for drug compounds and are available in the literature.

The term "cancer", as used herein, may include but is not limited to: biliary tract cancer; bladder cancer; brain cancer including glioblastomas and medulloblastomas; breast cancer; cervical cancer; choriocarcinoma; colon cancer; endometrial cancer; esophageal cancer; gastric cancer; hematological neoplasms including acute lymphocytic and myelogenous leukemia; multiple myeloma; AIDS-associated leukemias and adult T-cell leukemia lymphoma; intraepithelial neoplasms including Bowen's disease and Paget's disease; liver cancer; lung cancer; lymphomas including Hodgkin's disease and lymphocytic lymphomas; neuroblastomas; oral cancer including squamous cell carcinoma; ovarian cancer including those arising from epithelial cells, stromal cells, germ cells and mesenchymal cells; pancreatic cancer; prostate cancer; rectal cancer; sarcomas including leiomyosarcoma, rhabdomyosarcoma, liposarcoma, fibrosarcoma, and osteosarcoma; skin cancer including melanoma, Kaposi's sarcoma, basocellular cancer, and squamous cell cancer; testicular cancer including germinal tumors such as seminoma, non-seminoma (teratomas, choriocarcinomas), stromal tumors, and germ cell tumors; thyroid cancer including thyroid adenocarcinoma and medullar carcinoma; and renal cancer including adenocarcinoma and Wilms tumor. Preferred cancers are; breast, prostate, lung, ovarian, colorectal, and brain cancer.

The term "cancer treatment" as described herein, may include but is not limited to: chemotherapy, radiotherapy, adjuvant therapy, or any combination of the aforementioned methods. Aspects of treatment that may vary include, but are not limited to: dosages, timing of administration, or duration or therapy; and may or may not be combined with other treatments, which may also vary in dosage, timing, or duration. Another treatment for cancer is surgery, which can be utilized either alone or in combination with any of the aforementioned treatment methods. One of ordinary skill in the medical arts may determine an appropriate treatment.

An "agent for prevention of cancer or tumorigenesis" means any agent that counteracts any process associated with cancer or tumorigenesis described herein. For example, an agent that interacts with (e.g. binds to) to MGFR thereby reducing or preventing interaction, with MGFR, of an agent that promotes tumorigenesis by its interaction with MGFR.

An "agent that reduces cleavage of a cell surface receptor interchain binding region" as used herein is any composition that prevents or reduces cleavage of the MUC1 receptor between the MGFR and the IBR that would otherwise occur in the absence of the agent. Cleavage of the receptor between the MGFR and the IBR can be

5   caused by activity of enzymes that are membrane-associated or soluble. Some of these enzymes are directly responsible for cleavage. Other enzymes can affect cleavage, (e.g. prevent cleavage at a particular location) by modifying MUC1 with sugar groups or phosphates that mask a recognition epitope associated with cleavage. Other enzymes can promote cleavage at a particular location by modifying MUC1 with sugar groups or

10   phosphates that create a recognition motif for cleavage at that location. One way to select agents that reduce cleavage of a cell surface receptor IBR is to first identify enzymes that affect cleavage as described above, and screen agents, and their analogs, for their ability to alter the activity of those enzymes. Another way is to test agents that are known to affect the activity of similar enzymes (e.g. from the same family) for their

15   ability to alter the site of cleavage of MUC1, and to similarly test analogs of these agents. Alternatively, agents are screened in a cell-free assay containing the enzyme and MUC1 receptors, and the the rate or position of cleavage measured by antibody probing, Polymerase Chain Reaction (PCR), or the like. Alternatively, without first identifying enzymes that affect MUC1, agents are screened against cells that present MUC1 for the

20   agents' ability to alter cleavage site or the rate of cleavage of MUC1. For example, agents can be screened in an assay containing whole cells that present MUC1 and aggregation potential of the cell supernatant can be measured, an indication of the amount of IBR that remains attached to the cleaved portion of MUC1, i.e. the degree of cleavage between MGFR and IBR. In another technique, agents can be screened in an

25   assay containing whole cells that present MUC1, the supernatant removed, and the cell remain tested for accessibility of the MGFR portion, e.g. using a labeled antibody to the MGFR. Agents can be identified from commercially available sources such as molecular libraries, or rationally designed based on known agents having the same functional capacity and tested for activity using the screening assays.

30   An "agent that reduces cleavage of the MUC1 receptor" is any composition that prevents or reduces cleavage of the MUC1 receptor at any location. Such an agent can be used to treat a subject having cancer or at risk for developing cancer because if cleavage is prevented, then the accessibility of the MGFR, a functional receptor

associated with cancer, is reduced or prevented. Such agents can be selected by exposing cells to a candidate agent and determine, in the supernatant, the amount of cleaved MUC1 receptor, relative to a control.

A subject, as used herein, refers to any mammal (preferably, a human), and preferably a mammal that may be susceptible to tumorigenesis or cancer associated with the abherrant expression of MUC1. Examples include a human, non-human primate, cow, horse, pig, sheep, goat, dog, or cat. Generally, the invention is directed toward use with humans.

The samples used herein are any body tissue or body fluid sample obtained from a subject. Preferred are body fluids, for example lymph, saliva, blood, urine, and the like. Blood is most preferred. Samples of tissue and/or cells for use in the various methods described herein can be obtained through standard methods including, but not limited to: tissue biopsy, including punch biopsy and cell scraping, needle biopsy, and collection of blood or other bodily fluids by aspiration or other methods.

The following patent applications and publications are incorporated herein by reference: international patent application serial no. PCT/US00/01997, filed 01/25/00, entitled "Rapid and Sensitive Detection of Aberrant Protein Aggregation in Neurodegenerative Diseases", published as no. WO 00/43791, international patent application serial no. PCT/US00/01504, filed 01/21/00, entitled "Assays involving Colloids and Non-Colloidal Structures", published 07/27/00 as international patent publication no. WO 00/34783, U.S. patent application serial no. 09/631,818, filed 08/03/00, entitled "Rapid and Sensitive Detection of Protein Aggregation", a U.S. provisional patent application by Bamdad, et al., serial no. 60/248,865, filed 11/15/00, entitled "Endostatin-Like Angiogenesis Inhibition,"and a U.S. Utility Application Application of same title filed 11/15 2001.

The present invention involves, generally, novel molecular targets for drug screening, therapeutics and diagnostics related to cancers that are characterized by the aberrant expression of a class of cell surface receptors characterized by interchain binding regions. One such set of cancers are those characterized by the aberrant expression of MUC1. Much of the description of the invention herein involves cells that aberrantly express MUC1. It is to be understood that in these instances the description is to be considered exemplary, and that the principles of the invention apply to other cell surface receptors that function by a similar mechanism. With the disclosure herein, those

of ordinary skill in the art will readily be able to identify other cell surface receptors that function by this or a similar mechanism, and to apply the invention to those cancers characterized by aberrant expression of receptors. The invention is based on a novel mechanism involving cell surface receptors that have regions that self-aggregate,

5   exemplified by MUC1, which was elucidated by the inventors.

MUC1 comprises several regions termed herein as follows, recited in an order starting from the region closest to the cell surface and progressing away from the cell. In at least one U.S. provisional patent application ("earlier application(s)") filed by the same inventors, entitled "Tumor Markers and Drug Screening for Tumorogenesis

10   Inhibition", relating to MUC1 diagnostics and other techniques, at least one region of MUC 1 was defined differently. It is to be understood that the following definition supercedes. Those of ordinary skill in the art will understand the invention in all its aspects from the description of portions of MUC1 referred to differently in the earlier application(s) and in the current application, and the relation of the earlier application(s)

15   to this application. (1) the PSMGFRwas referred to in the earlier application(s) as an FLR region or peptide); (2) the PSIBR was referred to in the earlier application(s) as a CM region or peptide. The basic structure of the MUC1 receptor is illustrated in FIG.1. The receptor, as illustrated comprises: 1) cytoplasmic tail; 2) transmembrane section; 3) MGFR; 4) IBR, and 5) repeats.

20   One aspect of the present invention features the discovery that a specific region of the MUC1 receptor, i.e., the IBR, binds strongly to identical regions of other MUC 1 molecules. That is, the MUC1 receptor has the ability to aggregate (i.e. self-aggregate) with other MUC1 receptors via the IBR of the respective receptors. This self-aggregation may contribute to MUC1 receptor clustering, observed in healthy cells. The

25   discovery that the IBR portion of the MUC1 receptor self-aggregates is consistent with the following mechanistic model for which the inventors present supporting evidence. Mechanistic model: (1) receptor clustering is associated with the healthy state because the aggregated IBR portions block access of ligands, such as growth factors, modifying enzymes and the like to the neighboring extracellular portions of the MUC1 receptor that

30   act as the functional receptor; clustering also blocks access of intracellular tails to intracellular modifying enzymes and signaling ligands; (2) when the MUC1 receptor is cleaved at a position that releases the IBR, the critical force that keeps the receptors clustered is lost and receptors are free to migrate within the cell membrane or interact

with modifying enzymes, secreted ligands such as activating ligands or growth factors or other cell surface receptors; these interactions could involve a new, inductive multimerization state, such as dimerization, that triggers a cell proliferation signaling cascade.

Cleavage of MUC1 may occur at a site at or near the C-terminal boundary of the IBR in tumor or cancer cells (between the cell and the IBR), releasing the IBR from the cell. Alternatively, cleavage of MUC1 may occur within the IBR itself to cause sufficient disrupting of the IBRs such that the ability to self-aggregate is lost with the result that the MGFR becomes accessible to agents or ligands. As described in Example 1b, the addition of 9 amino acids of the IBR region to a peptide from the MGFR region (which does not self-aggregate), confers some ability to self-aggregate. Alternatively, loss of aggregation of MUC1 receptors need not necessarily be the result of cleavage. For example, an IBR can be absent as a result of alternative splicing of the MUC1 gene.

Before the present invention, a ligand(s) for the MUC1 receptor had not been conclusively identified. Research articles suggested that the shed portion of the MUC1 receptor becomes a ligand for the portion of the receptor that remains attached to the cell surface after cleavage. In the present invention, this hypothesis was tested, and it was determined not to be the case. Further, it was determined that altered sites of cleavage of the MUC1 receptor could result in altered function. In a colorimetric colloid binding assay (described in PCT/US00/01997, referenced above, as well as some other assays of the above-incorporated patent applications/publications) various fragments of the MUC1 receptor were tested for their ability to interact with each other fragment of MUC1 as well as for their ability to self-aggregate. We found that one portion of the receptor, fairly close to the cell surface, aggregated with itself in a high affinity interaction. This suggested that this portion of the MUC1 receptor, which we termed the interchain binding region, kept the receptors tightly clustered in a healthy cell, and that enzyme cleavage of MUC1 at a site that released the IBR, would cause the receptors not remain clustered. This clustering may affect cell signaling in two ways. First, the clustering of the receptors on the cell surface may restrict access to portions of the receptor that are binding sites for ligands such as modifying enzymes or growth factors. Secondly, as is appreciated by those skilled in the art, the intracellular portions (cytoplasmic tails) of many cell surface receptors are involved in signaling cascades that control programmed cell growth (proliferation) as well as programmed cell death (apoptosis). Receptors that

are tightly clustered on the cell surface also have clustered cytoplasmic tails within the cell, which may prevent them from interacting with intracellular proteins involved in intracellular signaling.

In some cases, the MUC1 receptor may be cleaved to release the IBR, from the cell surface. Alternatively, cleavage can result in a release of a sufficient portion of the IBR that causes the MUC1 receptor to lose the ability to self-aggregate. Loss of aggregation of MUC1 may have several ramifications. Release of the IBR or sufficient portion of the IBR from the cell surface allows the receptors to evenly distribute on the cell surface, leaving the cytoplasmic tails free to associate with intracellular signaling proteins. External agents, such as modifying enzymes and/or activating ligands, are then able to bind to the remaining extracellular portion of the receptor and induce disease-associated signals, either via a change in the multimerization state, i.e., inductive multimerization, or as an induced conformational change. As is appreciated by those of ordinary skill in the art, ligands such as growth factors and hormones often induce receptor dimerization which triggers, in turn, an intracellular signaling cascade.

Cell proliferation may result from accessibility of the MGFR portion to an activating ligand which can interact with the MGFR portion. For example, the self-aggregating IBR of the MUC1 receptor may form a dense reticulum which sterically prevents a ligand such as a growth factor from intreacting with the MGFR portion of the receptor, which is proximal to the cell relative to the IBR. In a cancerous or tumor cell, this reticulum may be lost, allowing ligand interaction with the MGFR.

The above mechanistic model is consistent with a mechanism whereby the portion of the MUC1 receptor, that remains attached to the cell surface after shedding of the IBR region, i.e. the MGFR, functions as a receptor for ligands that trigger cell proliferation. Evidence is also presented herein that indicates that this portion of the receptor is enzyme modified before it is able to be recognized by at least one of its ligands (See Example 8). This mechanism is demonstrated herein with a showing that: (a) an interaction between a ligand and this portion of the MUC1 receptor (MGFR), which dimerizes the receptor, triggers cell proliferation; and (b) blocking the interaction of this portion of the MUC1 receptor (MGFR) with its ligand(s), blocks cell proliferation. When tumor cell lines, in which the MUC1 receptor is homogeneously expressed across the entire cell surface, are treated with an IgG antibody raised against the MGFR portion of the MUC1 receptor, the rate of cell proliferation is greatly

enhanced, see Fig. 5. Since IgG antibodies are bivalent, i.e. one antibody simultaneously binds to two adjacent MGFR portions on the cell surface, these results demonstrate that the antibody acts as an activating ligand, mimicking the effect of a growth factor, which dimerizes MGFR portions, and thus triggers a cell proliferation signaling cascade which

5   is consistent with signaling via the cytoplasmic tails of the receptors. This finding leads to two conclusions. First, an activating ligand(s) that binds to the MGFR portion of the MUC1 receptor causes inductive multimerization of the receptor. Secondly, an effective therapeutic strategy is therefore to block the MGFR portion of the receptor with a monomeric composition, thus preventing inductive multimerization and subsequent

10 signaling cascades. For example, a single chain, or monovalent, antibody raised against the MGFR portion of the MUC1 receptor would function as an effective anti-cancer therapeutic. Another therapeutic strategy is to block the activity of enzymes that modify the receptor, which may be required for some ligand binding

The inventors have also discovered that cells that overexpress the MUC1 receptor

15 also have increased levels of a ligand(s) that dimerizes the MGFR present in the lysates and supernatants of these cells, see Example 3b and Fig. 8A-D for details. In the colloid-colloid interaction experiment, described in Example 3b, ligands that simultaneously bind more than one colloid-immobilized receptor, i.e. dimerize, cause a solution color change from pink to blue. Gold colloids that presented synthetic peptides derived from

20 the MGFR portion of the MUC1 receptor (His-PSMGFR) were incubated with lysates/supernatants from various cells lines known to overexpress, express, or not express the MUC1 receptor. Lysates from HTB-133 (T47D) cells, which overexpresses MUC1 (see Table 2), caused colloid suspensions to turn blue within 15 minutes, indicating a high concentration of a ligand(s) in the lysate that interacts with the colloid-

25 immobilized MGFR-derived peptides. In experiments with lysates from other cell lines, the rate of color change of the colloid solution, which indicates the amount of ligand present, correlated to the degree of expression of the MUC1 receptor in those cell lines with cells.

More than one species may be a physiologically relevant ligand for this portion of

30 the MUC1 receptor. Enzymes that modify the receptor may be relevant ligands of this portion of the receptor. For example, one ligand may bind monomerically to an unmodified MGFR portion of the MUC1 receptor, while another ligand, with a different function, such as inductive multimerization, may recognize an enzyme-modified version

of the receptor. Because the experiment described above, Example 3b, was performed in cell lysate/supernatants, it is important to note that several receptor-ligand interactions, including enzymatic modifications to the receptor, may be taking place, wherein only the ligand(s) dimerization (or multimerization) of the MGFR portion, results in a solution

5   color change. In an experiment similar to Example 3b, (Example 8) the enzyme inhibitor, PMSF was added to the lysate prior to the introduction of the colloids bearing the synthetic peptide His-PSMGFR, see Table 1 SEQ ID # 2 . Referring now to Figure 20, solutions that contained PMSF did not undergo the solution color change. This result is consistent with a mechanism in which the MGFR portion of the MUC1 receptor is first

10   enzyme mofdified before it is recognized by the ligand(s) that dimerize or multimerize the receptor

        The inventors reasoned that prior attempts by others to identify ligands for the MUC1 receptor were hampered by the self-aggregation properties of the receptor. Therefore, only the MGFR portion of the receptor was used as bait to fish ligands out of

15   lysates and supernatants. The His-PSMGFR sequence of Table 1, was immobilized on NTA-nickel beads via the histidine tag of the peptide, the beads were then incubated with lysates and supernatants from a variety of cell types, including cancer cell lines that overexpress MUC1. Enzyme inhibitors such as PMSF were added to some of the lysates and supernatants to circumvent problems of alternative ligands binding to modified

20   versions of the peptide. Following incubation of the cell supernatants with the PSMGFR-presenting beads, the beads were washed, then the peptide-ligand complexes eluted from the NTA-Nickel beads by adding excess imidazole. Captured ligands from the probe peptides, eluates, were separated using standard SDS-PAGE methods. Protein bands were excised from the gels and analyzed to identify the target ligands. Standard

25   methods for protein analysis including peptide micro sequencing and tandem mass spec were used in these studies. Other methods can be used to identify MUC1 ligands, including ligand fishing with beads, MALDI mass spec, and the like.

        Accordingly, another aspect of the invention involves the identification of ligands, derived from lysates from a cell line selected from the group consisting of HTB-

30   133, CRL-1504, and CRL-1500, that bind to the MGFR portion of the MUC1 cell surface receptor, see Figs. 9 and 10. In some embodiments, the ligands may include sequence(s) from: Metastasis Inhibition Factor NM23, 14-3-3, Cathepsin D, annexin, Beta lipotropin (Beta-LPH), beta-melanotropin or Beta-MSH. In other embodiments, the

biomolecule that binds to the MGFR portion may be a cleavage product of proopiomelanocortin (POMC). In all embodiments, the preferred cell surface receptor is MUC1. In one embodiment, the MGFR portion includes some or all of the sequence from the PSMGFR peptide (SEQ ID NO: 7). These ligands may exist in a multimeric

5   state including dimers, tetramers, or complexes containing some or all of these ligand species. In one aspect, the invention involves modification and use of the above species as anti-cancer agents.

In one embodiment the ligand is a 23kD protein. In another embodiment, the ligand is approximately 17kD. In a preferred embodiment, the ligand identified is a

10   protein that migrates through a gel with an apparent molecular weight of 35kD. Since this species is much more apparent when the protease inhibitor PMSF is not added to the lysate (Fig. 10), this protein may be an enzyme that modifies the MUC1 receptor or a ligand that recognizes the unmodified version of the receptor. Experiments were performed as described in more detail below in Example 4b to characterize these species.

15   Peptide sequences contained within both the 17 kD and the 23 kD bands (PMSF added to lysate) corresponded to a protein known as Metastasis Inhibition Factor NM23, which has been implicated in both the promotion and inhibition of metastasis of human cancers. Whether the role of NM23 is a tumor supressor or promoter may depend on the type of cancer. In ovarian, colon and neuroblastoma tumors, NM23 overexpression has

20   been linked to a more malignant phenotype (Schneider J, Romero H, Ruiz R, Centeno MM, Rodriguez-Escudero FJ, "NM23 expression in advanced and borderline ovarian carcinoma", *Anticancer Res*, 1996; 16(3A): 1197-202). However, breast cancer studies indicate that reduced expression of NM23 correlates with poor prognosis (Mao H, Liu H, Fu X, Fang Z, Abrams J, Worsham MJ, "Loss of NM23 expression predicts distal

25   metastases and poorer survival for breast cancer", Int J Oncol 2001 Mar;18(3):587-91). Because NM23 exists as a hexamer, in MUC1-presenting cells, it may function to hold MUC1 receptors in a clustered configuration to restrict access of the MGFR to modifying and activating ligands. The sequences that were identified from the protein gel band described in Figures 9 and 10 and that also exist in Metastasis Inhibition Factor

30   NM23 are shown below in Table 4

Peptide sequences that are associated with the 35 kD gel band (PMSF NOT added to lysate) corresponded to more than one protein species, including 14-3-3, which is a signaling protein implicated in many cancers, and cathepsin D, which is a protease

and is also implicated in tumor progression. 14-3-3 exists as a dimer and can simultaneously bind to two, identical phospho-serine peptides. This protein has been shown to be involved in intracellular signaling, but particularly relevant to this invention is the fact that 14-3-3 has been shown to be secreted by some cell types, including

5   dendritic cells. Ligands that bind to the MGFR portion of the receptor to induce inductive multimerization would tend to be secreted factors. This protein would dimerize the MUC1 receptor to trigger cell proliferation, which is consistent with the mechanism presented herein. Cathepsin D is a protease and may be involved in the cleavage of the MUC1 receptor.

10   Yet another aspect of the invention involves the identification of other ligands, also derived from supernatants from a cell line selected from the group consisting of HTB-133, CRL-1504, and CRL-1500, that bind to the MGFR portion of the MUC1 cell surface receptor. In one embodiment, the ligand identified is a protein that migrates through a gel with an apparent molecular weight of 55kD. In another embodiment, the

15   ligand is approximately 70kD; 80kD; or 100kD. In a particular embodiment, the ligand present in cell supernatants is a 13kD protein, see Fig. 9., which migrates though gel with apparent molecular weight of 13kD. The protein with an apparent molecular weight of about 13 kD appears upon polyacrylamide gel electrophoresis as a smeared band which may indicate that the protein is glycosylated, enzyme modified, or that the band contains

20   more than one protein species. Using mass spec and maldi mass spec techniques, combined with homolgy to peptide sequences in the Genbank database, it was determined that two fragments derived from the 13 kD corresponded with a high degree of homology to beta-lipotropin (Odell W, Wolfsen A, Bachelot I, and Hirose F, (1979) "Ectopic production of lipotropin by cancer" *The American Journal of Medicine* 66; pgs.

25   631-638), which was previously known as beta-MSH (beta-melanotropin). Beta-lipotropin (beta- LPH: 98 amino acids or about 10 kd) and ACTH (aa' 130-169) are cleavage products of proopiomelanocortin (Publisher Williams & Wilkins, chapter authors: Faye W, Lemke T, Williams D, Text book – Principles of Medicinal Chemistry; Fourth edition 1995) (POMC: 260 amino acids). Because these peptides are

30   glycosylated, their apparent molecular weights can be altered from their actual molecular weights. These cell surface receptor binding ligands can be purified or produced using techniques known to those of skill in the art. It is also possible that in certain situations an external ligand does not bind to and dimerize the MGFR portions of the MUC1

receptor to trigger cell proliferation, but rather the receptors become covalently coupled to each other, for example by an enzyme that covalently links the two. One way this could be accomplished is by an enzyme that attaches an entity, such as a sugar group, to both receptors.

5   Because the portion of the MUC1 receptor that self-aggregates (IBR), and in doing so may protect intracellular signaling sequences from participating in signaling cascades that induce proliferation, can be cleaved and shed from the cell surface, it can be beneficial to identify small molecules that interact with the MGFR portion of MUC1 that remains cell-attached (MGFR). These small molecules that bind to the portion of

10   MUC 1 that remains cell-attached (MGFR) can then be used in two ways. First, they can be used to block the remaining, cell-attached portion of the MUC1 receptor so that it cannot interact with activating ligands that induce proliferation and metastasis. For example, a ligand to the cell-attached portion of MUC 1 may effect inductive multimerization of the receptor, causing a signaling cascade inside the cell. Blocking

15   binding of the ligand to the MGFR region can inhibit the signaling cascade that causes proliferation. Secondly, as discussed in more detail below, the small molecules can be polymerized or attached to dendrimers to artificially cause preventative clustering the cell-attached MGFR portions of the MUC1 receptors and thus shield the cytoplasmic tails from interaction with intracellular signaling proteins.

20   The findings of the invention have important implications for diagnostic and therapeutic procedures. For instance our finding that a fragment of the MUC1 receptor that is close to the cell surface aggregates with itself, indicates that the position of enzyme cleavage is associated with receptor clustering, accessibility of adjacent portions of the receptor to putative ligands, and thus cancer. Agents that modulate the activity of

25   this enzyme may be potent anti-cancer agents. Additionally, an early diagnostic test for cancers that aberrantly express MUC1 may be based on detecting the portion of MUC1 that self-aggregates (IBR) circulating in bodily fluids. This portion of MUC1 includes part or all of the PSIBR (sequence shown in Table 1). Agents that bind to the portion (some or all of the PSMGFR sequence) of MUC1 that remains attached to the cell

30   surface after the release of the portion that self-aggregates (IBR – some or all of the PSIBR sequence) may be potent anti-cancer drugs. In addition, agents that block binding of the natural ligand to the remaining portion after the release of the IBR, may also be useful as anticancer drugs. Drug candidates that target portions of the MGFR, ie.,

sequences including some or all of the amino acids contained within the PSMGFR, of the receptor can be used either as monomers, to block the interaction of the MGFR portion of MUC1 with extracellular agents or biomolecules, or as a polymer, dendrimer, etc. to both block the interaction with external biomolecules and to artificially cluster the MGFRs. Another alternative agent, which can be used to artificially cluster the MGFRs is an IgM antibody raised against the MGFR or PSMGFR. This artifically-induced clustering may serve to keep the cytoplasmic tails clustered to prevent interaction with intracellular signaling agents, thereby effecting preventative clustering.

One aspect of the invention involves s novel drug screening assays, that identify therapeutics that interfere with the proliferation of tumor cells that aberrantly express MUC1. The drug screen makes use of the new molecular target for cancer that is disclosed herein. Another aspect of the invention involves therapeutics identified by the drug screen. Yet another aspect of the invention involves methods for diagnosing MUC1$^+$ cancers, which is based upon the mechanism elucidated by the inventors.

One embodiment of the invention involves a drug screening assay which can rapidly identify agents that interrupt the interaction between the MGFR and its ligand(s) and thus can be used as cancer therapeutics, (see Example 5a and Fig. 12 for details). The fact that an activating ligand(s) that binds to the MGFR portion of the MUC1 receptor can result in inductive multimerization of the receptor, allows us to construct a convenient drug-screening assay to identify compounds that inhibit this interaction or inhibit enzymes that modify the MGFR portion required for ligand binding and thus inhibit the proliferation signal. In one assay of the invention, synthetic peptides which include much or all of the MGFR sequence are attached to nanoparticles such as gold colloids. Gold colloids have the intrinsic optical property that when in a disperse, homogeneous solution, the solution appears pink, but when the colloids are forced into close proximity, the solution turns blue. When cell lysates or supernatants, which contain a ligand(s) that dimerizes the colloid-attached peptides, are added, the colloids becomes aggregated and the solution turns blue. Drug candidates that interrupt this ligand-receptor interaction are easily identified because they cause the solution to remain pink.

As discussed above, it appears that the ligand that binds to the MGFR portion of the receptor to trigger inductive multimerization may recognize an enzyme-modified form of the receptor. Therefore the above-described drug screening assay can identify compounds that: a) inhibit enzyme modification of the MGFR portion of the MUC1

receptor; b) bind to the MGFR region and block its interaction with an activating ligand of the MGFR portion; or c) bind to an activating ligand, such as a growth factor and block its interaction with the MGFR portion. Drugs that act according to (a) or (b) are predicted to selectively inhibit MUC1-dependent cell proliferation, whereas drugs that act according to (c), on agents such as growth factors, are expected to inhibit proliferation of a variety of cell types, see Examples 5a-d.

Drugs that have been found by the above protocol to disrupt an interaction between the MGFR portion of the MUC1 receptor and its ligand(s) are described in much greater detail below and include, for example, calcimycin, fusaric acid, L-α-methyl-dopa, butylindazone, NS1619 or etomoxir. Additional drugs are described in greater detail in applicant's co-pending U.S. Provisional Patent Applications Serial Nos. 60/317,302 and 60/317,314, both filed on September 5, 2001 and entitled COMPOSITIONS AND METHODS OF TREATMENT OF CANCER.

Agents so identified may be potent anti-cancer agents either in monomeric form or as polymers or dendrimers. Drug libraries and peptide libraries can be screened for molecules that inhibit binding of the ligand to its target.

Alternatively, standard methods can also be used to identify agents that interrupt the interaction between the MGFR and its ligand(s). These methods can be used to identify agents that bind: (1) to the MGFR portion of the MUC1 receptor, or (2) to one or more of its ligands. These methods include but are not limited to phage display methods, yeast two-hybrid system, sandwich assays, surface plasmon resonance-based assays, antibody-based assays, peptide bead assays for testing with drug libraries, bead assays, GFP-reporter assays, and the like. Ligands to the MGFR portion of the MUC1 receptor can be identified by a number of methods including using a peptide whose sequence corresponds to some or all of the MGFR, ie. the PSMGFR peptide (Sequence ID 7), as bait to fish out ligands. Another way to do this is to attach a signaling entity, such as GFP (green fluorescent protein), directly or indirectly to the ligand and attach the receptor-derived peptide to a solid support. Compounds that interfere with the interaction will cause a loss of signal.

As an alternative to the natural ligand competition assays described above, direct binding assays can be employed to identify drug agents able to interact with the MGFR. Small molecules that bind to the remaining extracellular portion of cleaved MUC1

(MGFR region:exemplified by most or all of the PSMGFR peptide sequence), can be identified using standard methods (MALDI, western blotting, gel electrophoresis, ELISA, etc) or using colloid-colloid or colloid-bead binding assays. For example, in one embodiment, small molecules can be synthesized on beads or attached to colloids either

5 by attachment to a thiol and direct incorporation into a SAM on the colloid or by EDC/NHS coupling of molecules containing a primary amine. A histidine-tagged peptide including the desired MUC1 (e.g. His-PSMGFR) sequence can be bound to colloids and assayed for interaction with a second set of colloids presenting a candidate drug. A color change from pink to blue would indicate an interaction between the

10 MUC1 peptide and the small molecule. Alternatively, the MUC1-bearing colloids can be assayed for an interaction with a small molecule attached to a bead. Red coloration of the bead surface would signify that the MUC1 peptide bound to the bead-immobilized small molecule.

Any drugs or small molecules identified as binding to the MUC1 sequence can

15 potentially be used to block binding of the remaining extracellular portion of cleaved MUC1 to its natural ligand, and can potentially inhibit cancer growth.

In another embodiment, the above-described competition assays are employed to identify "second generation" drug candidates by assaying such candidates for their ability to disrupt an interaction between the MGFR portion of the MUC1 receptor and a

20 synthetic ligand found, according to the inventive methods described above, to bind to the MGFR, e.g. calcimycin, fusaric acid, L-α-methyl-dopa, butylindazone, NS1619 or etomoxir. In this way, drugs that bind the MGFR with higher affinity than the "first generation" drug can be identified. For example, in performing the drug screen, a synthetic ligand such as calcimycin, fusaric acid, L-α-methyl-dopa, butylindazone,

25 NS1619 or etomoxir is modified to facilitate attachment to surfaces, such as colloids. A peptide derived from the MGFR region of the MUC1 receptor, such as the His-PSMGFR, is attached to a second set of colloids. Direct binding between the synthetic ligand and the MGFR peptide is confirmed, a library of drug candidates are then assayed for their ability to disrupt the interaction. It is not intended that this aspect of the

30 invention be limited to the competition techniques or assays explicitly described herein. Several techniques, including standard methods, could potentially be used to detect

binding between the MGFR and a synthetic ligand or drug, then competitive inhibition of binding by a drug candidate.

Another aspect of the invention is a drug screening assay for identification of drugs that can be useful for prevention and/or treatment of cancer by altering the cleavage state of MUC 1 receptors on cells. In such assays, described in more detail below, cultured cells are exposed to candidate drugs. The cleavage state of MUC 1 in the cells is determined, optionally as a function of time and/or dosage or other conditions involving exposure to the drugs. These cells can be derived from a particular patient, or can be tumor-associated or non-tumor-associated cell lines. Customized therapeutic protocols can be determined for a particular patient in this manner. The invention involves, in one aspect, treating a patient with a drug, as discused in greater detail below, shown to affect the cleavage state of MUC 1 of the patient's cells in a manner that prevents, inhibits, or reverses cancer.

Because it is suspected that the incorrect cleavage of MUC1 on the surface of the cell causes the cascade leading to proliferation and tumorigenesis, it would be advantageous to test candidate drugs in a whole cell assay for their ability to affect enzyme cleavage or the position of enzyme cleavage of MUC1. Drugs can be screened for their ability to effect MUC1 cleavage, either directly or indirectly. This can enable the identification of upstream effectors of MUC1 cleavage. Cells or tissue samples can be assayed for MUC1 cleavage potential in several ways. For example, cells or a tissue sample can be treated with a drug candidate and grown for some period of time. Colloids bearing an antibody, natural ligand, or small molecule that binds to either the cleaved portion of MUC1, or the remaining extracellular portion (plus or minus a signaling moiety) can be added and allowed to bind to the cells or tissue sample. The expression of cleaved MUC1 or uncleaved MUC1 on the cell surface as compared to a control sample, not treated with the candidate drug, would indicate whether the drug candidate effected MUC1 expression or cleavage.

Alternatively, MUC1 expressing cells can be assayed for MUC1 cleavage in the presence of a drug candidate by testing the surrounding cell growth media for the presence of cleaved MUC1 or the potential of the cleaved portion to self-aggregate. For example, cells expressing MUC1 would be treated with a drug candidate suspected of interfering with enzyme cleavage. After some incubation period, the cell media would be removed and tested for its aggregation potential, i.e. to determine whether the self-

aggregating portion of MUC1 was contained within the shed fragment. The aggregation potential of peptides released into the cell media is tested by adding colloids bearing an antibody to a sequence distal from the self-aggregating portion, but not a repeat sequence. In this way, antibody-presenting colloids would attach to upstream regions of

5 MUC1. If the self-aggregating region was also attached to the released fragment, then this would cause aggregation of the attached colloids and a solution change from pink to blue would result. Accordingly, one aspect of the invention provides a composition (drug or agent) that, in contrast to preventing inductive multimerization of cleaved or modified MUC1 receptors as discussed previously, instead prevents disease-associated

10 MUC 1 cleavage or modification itself. MUC 1 cleavage is an enzymatic process. The determination of the mechanism involved by the inventors can lead to identification of a drug that inhibits disease-associated cleavage.

As mentioned above, another aspect of the invention provides an agent that binds together MGFR portions of MUC 1 following disease-associated cleavage to effect

15 preventative clustering of the receptors. The agent can be any species that includes multiple sites each able to bind to a MFGR portion, and immobilized with respect to each other. E.g. a polymer or dendrimer or other continuous entity can include multiple sites each able to bind to a MGFR portion, causing clustering of these portions or other structural constraint that inhibits their association with factors that promote cell

20 proliferation. Alternatively, IgM-type monoclonal or polyclonal antibodies raised against the MGFR or PSMGFR could be utilizied. Each anti-MGFR IgM antibody could be able to aggregate ten MGFRs on the cell surface to form preventative clusters.

In addition, some or all of the above-identified ligand species that bind to the MGFR can be modified to allow the ligands to act as a targeted delivery agent by

25 attaching a cytotoxic drug or other agent (e.g. a radioactive substance) able to selectively kill cells to which the ligands become immobilized. In addition, synthetic ligands, such as calcimycin, fusaric acid, L-α-methyl-dopa, butylindazone, NS1619 or etomoxir, discussed in more detail below, that were found to bind to the MGFR portion of the MUC1 receptor can similarly be modified with other therapeutic agents. In this way,

30 such a therapeutic can be directed to the tumor cells. For example, an agent that binds to the MGFR region of the MUC1 receptor can be modified with a radioactive substance to destroy tumor cells that aberrantly express the MUC1 receptor. Other toxic substances,

such as ricin, as well as other therapeutics, can be attached to agents that bind the
MGFR. Alternatively, identified ligand species that bind to the MGFR could be modified
to present a imaging agent for use in diagnostic imaging of MUC 1$^+$ tumors and
metastases. Such ligands can also, alternatively, be modified to act as drugs that can be
5   useful for prevention and/or treatment of cancer. In one embodiment, a ligand, which in
its unmodified form binds to multiple MGFRs causing inductive multimerization, is
modified to remove or de-activate all but one of its active binding sites for MGFR, such
that each modified ligand is able to bind to only a single receptor. In another
embodiment, individual ligand molecules/peptides are modified such that they are
10   immobilized with respect to additional ligand molecules/peptides also able to bind
MGFR, e.g. through covalent coupling, non-covalent coupling, co-immobilization with
respect to a substrate, etc., such that the modified, multi-unit ligand is able th effect
preventative clustering of the receptors to which it binds.

Identification of the ligand(s) for the portion of MUC1 that remains bound to the
15   cell after cleavage can allow, as discussed further below, for development of powerful
assays to screen for drugs that disrupt this interaction. Interaction of potential binding
partners with the extracellular portion of MUC1 that remains after cleavage can be
studied both by conventional techniques (western blotting, ELISA, MALDI, etc.) and
using our colloid-colloid color change assay or colloid-bead coloration assay. The
20   peptide sequence of the remaining extracellular portion of MUC1 can be attached to
beads or colloids via a histidine tag. Potential binding partners can be histidine-tagged
and attached to a second set of colloids (or beads) and assayed for binding to the colloid-
immobilized portion of MUC1. Alternatively, potential binding partners can be attached
to beads or colloids by EDC/NHS coupling or can be nonspecifically adsorbed to beads
25   for the assay. An interaction between the MUC1 peptide and the potential binding
partner can be detected by either a change in solution color (for the colloid-colloid assay)
or by agglomeration of the colloids onto the bead, causing the bead to appear red (for the
colloid-bead assay). An entire cDNA library can be screened using this technique in a
short period of time to identify the natural ligand of the remaining extracellular MUC1.
30   (see PCT/US00/01997, WO 00/34783, 09/631,818, and "Detection of Binding Species
with Colloidal and Non-Colloidal Structures", filed 11/15/00, incorporated above).

The discoveries presented herein: (1) that the IBR of MUC1 self-aggregates; (2)
that an antibody that dimerizes adjacent MGFR portions of the MUC1 receptor leads to

proliferation of MUC1 presenting tumor cells; and (3) that proliferation of MUC1 presenting tumor cells can be inhibited by treatment with agents that target the MGFR and block the MGFR against interaction with a ligand, are consistent with a mechanism in which, in a healthy cell, MUC1 cleavage occurs such that enough of the IBR remains

5   on the cell that MUC1 remains clustered, and the MGFR is inaccessible to ligands such as growth factors, and in a tumor cell, MUC1 cleavage occurs such that enough of the IBR is cleaved from the cell such that MUC1 does not remain clustered, and the MGFR is accessible to ligand interaction. This leads to diagnostics, provided by the present invention, in which the shed portion of MUC1 is analyzed to determine the degree of

10   IBR present.

The above-mentioned mechanistic model predicts that in a subject with a MUC1-dependent tumor or who is prone to developing such a tumor, the portion of the MUC1 receptor that is shed will contain the IBR region of the receptor, leaving the MGFR portion of the receptor accessible for interactions with ligands and growth factors. An

15   early diagnostic would consist of detecting the IBR region in the portion of the MUC1 receptor which is shed.

In one embodiment, loss of aggregation of MUC1 receptors can result from the "cleavage state" of MUC 1. "Cleavage state" defines the result of cleavage of MUC 1. The cleavage state will differ between a healthy cell and a cell with tumor potential. The

20   cleavage state determination can involve determining whether cleavage occurs in a manner such that the normal interaction between the IBRs of neighboring receptors is disrupted (these regions no longer remain bound to each other at the cell) and MUC 1 is free to spread across the cell surface. More specifically, determination of the cleavage state can include determining a site of cleavage of MUC 1, determining the identity of a

25   portion of MUC 1 that remains at a cell following cleavage, determining the identity of a portion of MUC 1 that is separated from the cell following cleavage ("cleaved or shed portion"), determining the accessibility of MGFR, or a combination.

In one embodiment, an assay is provided that can determine whether the MUC1 IBR remains fastened to the cell, or is separated from the cell upon cleavage. The

30   product of MUC1 cleavage from the cell is exposed to at least one surface adapted to bind the IBR and to another surface and/or a signaling entity. Generally, an assay as described in WO 00/43791 or WO 00/34783 can be used. In a specific example, antibodies to a portion of MUC1 that would remain fastened to the IBR if the IBR is

cleaved from the cell, such as antibodies to the repeats domain, are fastened to colloids. Exposure of these colloids to the MUC1 cleavage products will allow the IBR regions to self-aggregate which in turn will result in colloid/colloid aggregation (color change to blue) for cases in which the IBR portion is separated from the cell. This is because each

5   colloid will fasten to a MUC 1 region which is connected to an IBR which is fastened to another IBR which is in turn fastened to another colloid.

The discovery that tumor cells can be treated with an agent that binds to the MGFR of MUC 1, or a ligand of MGFR, in a manner that inhibits cell proliferation leads to the conclusion that, in a diseased cell (a cancerous cell or a cell with potential for

10   becoming cancerous), cleavage of MUC 1 occurs in a manner that allows MGFR to interact with at least one ligand in a manner that promotes tumorigenesis or cancer. Interaction with the ligand may be due to cleavage that disrupts binding between different MUC 1 molecules at the cell surface, either via separation from the cell of the IBR during cleavage, or cleavage within the IBR at a location that frees MUC 1

15   molecules from each other.

In one aspect of the invention, a diagnostic is provided wherein an amount of cleavage of a cell surface receptor IBR from the cell surface is determined. This involves the determination of the amount of cell surface receptor IBR that is separated from the cell surface upon cleavage, which can be relative to the level determined in past

20   samples, the level in control samples, or can be determined as a ratio of the amount of IBR to the amount of a constant region of the receptor in a sample. The constant region is any non-repeating sequence which is N-terminal to the boundary of the PSIBR, that is, this region is present in a 1:1 ratio with the PSIBR prior to cleavage – determination of the ratio of IBR to constant region subsequent to cleavage indicates the extent to which

25   the IBR is cleaved and separated from the cell. The amounts of various receptor regions may be determined with any type of binding assay, e.g. an antibody-binding assay. For example, antibodies that specifically bind to the constant region or the repeats may be attached to surfaces (e.g. magnetic beads) to preconcentrate shed MUC 1 receptors prior to determining levels of IBR present. Then, for example, after pre-concentration of

30   circulating MUC 1 receptors, antibodies to the IBR and antibodies to the constant region can be allowed to bind to the cleaved receptors, and determination of the ratio of binding of these antibodies reveals the ratio of IBR present relative to constant region present in the cleaved receptors, which in turn reveals the amount of cleavage that occured in a

manner that caused separation of the IBR from the cell. A ratio that displays a trend toward less than one (less than a 1:1 ratio of IBR relative to constant region present) for detecting IBR at a cell surface is an indicator of the presence of a tumor or the potential for the development of a tumor. A ratio that approaches 1:1 when detecting these

5   regions in shed receptors is likewise an indicator of cancer potential. This determination can indicate potential for tumor formation, existence of a tumor, progression of tumorigenesis, etc., and can thereby serve as a diagnostic and/or a evaluator of treatment for tumorigenesis

Another diagnostic aspect of the invention involves determining levels of shed

10 IBRs in sample from a subject. Methods for such determination can include determining the aggregation potential of the circulating shed receptors, for example via colloid-binding assays such as a colloid-colloid assay or a colloid bead assay (See above discussion and Examples, below). Alternative techniques involve determining the presence of the IBR using antibody probing assays, hybridization, PCR Reverse

15 Transcriptase PCR (rtPCR), Ligase Chain Reaction (LCR), cycling probe technology, etc. In a preferred embodiment of the invention, the cell surface receptor is MUC1.

The determination, in a blood sample, of the amount of cleaved receptor carrying IBR, either involving antibody binding ratios, colloid binding assays, or the like can be made on a bodily fluid sample, such as a blood sample and optionally compared with

20 other samples (e.g. to monitor the subject's progression of tumorigenesis or progression for treatment of the same) and/or controls.

Alternatively, biopsy specemins can be studied, or tissue can be studied interoperatively (e.g. tissue at a surgical site can be studied without removal of the tissue from the subject). In either of these studies, a primary indicator of tumorigenesis or

25 potential for tumorigenesis is the amount of MGFR at a cell surface accessible to interaction with external agents such as growth factors, etc. This determination can be made, for example, by determining the amount of an antibody to the MGFR region that binds to the sample, either using standard antibody binding study techniques, or by exposing the sample to colloids to which antibodies specific to the MGFR region have

30 been immobilized and determining binding of the colloids to the samples using techniques described in International patent publication numbers WO 00/34783 and WO 00/43791, referenced above. In another technique (perhaps more suited for an excised sample), antibodies to the MGFR region and to the IBR can be exposed to the sample

and a determination made of the ratio of binding of each to the sample. A healthy sample will exhibit little or no antibody binding to the MGFR region. A sample indicating tumerigenesis or potential for tumorigenesis will show a non-zero ratio of MGFR antibody binding to IBR antibody binding.

5   Whether shed MUC1 contains the IBR can be identified by methods known to those of ordinary skill in the art, including ELISA and colloidal assays as described in international patent application serial no. PCT/US00/01997, filed 01/25/00, entitled "Rapid and Sensitive Detection of Aberrant Protein Aggregation in Neurodegenerative Diseases", published as no. WO 00/43791, and U.S. patent application serial no.

10   09/631,818, filed 08/03/00, entitled "Rapid and Sensitive Detection of Protein Aggregation", by Bamdad et al. In a preferred embodiment of the invention, the cell surface receptor is MUC1.

Another aspect of the invention involves determining the site of cleavage of the MUC1 receptor from a cell surface (rather than the amount of IBR in a shed portion) in a

15   sample from a subject to evaluate cancer, or the potential to develop cancer in a subject. Determination of the site of cleavage will give information as to whether the IBR remains on the cell surface, or was shed from the cell surface, giving indication of cancer or tumorigenesis or the potential for either, as discussed above. Determining the site of cleavage can be accomplished by using enzyme-amplification methods such as PCR.

20   Specifically, using alternative primer sites in PCR amplification of shed MUC1 from subject's sample will indicate where cleavage occurred.

In one aspect of the invention, differences in pre- and post-treatment levels of cleaved cell surface receptor IBR, or cell surface receptor IBR at the surface of a cell, in cancer cells or tissues may be used to diagnose cancer in a subject or assess the

25   effectiveness of treatment in a cancer patient. In a preferred embodiment the cell surface receptor is MUC1.

Comparison of the levels of the above-mentioned regions with levels from subjects known to be free of cancer may allow determination of the presence of cancer in the subject. An example, although not intended to be limiting, is that a determination of

30   the presence of elevated levels of cleaved cell surface IBR in a sample from a subject, when compared to a level determined in samples from control subjects, may suggest the presence of cancer in the subject with elevated levels. Such methods of comparing levels of cancer-associated markers between a sample from a subject and a control sample for

diagnostic purposes would be understood by one of ordinary skill in the medical arts. Examples of such methods include Western blotting, ELISA, antibody precipitation, PCR, LCR, rtPCR, cycling probe technology, and colloidal assays as described in international patent application serial no. PCT/US00/01997, filed 01/25/00, entitled "Rapid and Sensitive Detection of Aberrant Protein Aggregation in Neurodegenerative Diseases", published as no. WO 00/43791, and U.S. patent application serial no. 09/631,818, filed 08/03/00, entitled "Rapid and Sensitive Detection of Protein Aggregation", by Bamdad et al. In a preferred embodiment the cell surface receptor is MUC1.

In another aspect of the invention, the cleavage state of MUC 1 can be used to determine progression or regression of a subject's cancer over time. The cleavage state also can be used to assess treatment parameters including, but not limited to: dosage, method of administration, timing of administration, and combination with other treatments as described herein.

Another aspect of the invention involves extremely early-stage cancer diagnosis. This aspect involves identification of patients who may be at risk for developing tumor or cancer associated with abnormal cleaveage of MUC1. These patients may not have developed tumors, but may exhibit a cleavage state indicative of a condition that can lead to cancer. In some instances, the subjects will already be undergoing treatment for cancer, while in other instances the subjects will be without present cancer treatment. A test for a genetic predisposition to cancers characterized by aberrant MUC1 expression of the invention is based on detecting genetic alterations in the MUC1 cleavage enzyme(s), over expression of MUC1 activating ligands, and/or overexpression of enzymes that modify the MGFR portion of the receptor.

The fact that elevated levels of cleaved MUC1 are found in the blood of cancer patients is the basis for a blood test for breast cancer, which is not described herein. MUC1 is cleaved by at least one enzyme and may be cleaved at more than one site before it is released into the blood stream. Several protease cleavage sites within the MUC1 sequence are predicted. Predicted sites for enzyme cleavage are at or near amino acid 540, 528, 530, 542, and 550 (numbers are as listed in Andrew Spicer *et al.*, J. Biol. Chem Vol 266 No. 23, 1991 pgs. 15099-15109; these amino acid numbers correspond to numbers 1100, 1088, 1090, 1102, 1110 of Genbank accession number P15941; PID G547937; Boshel M., et al, BBRC vol. 185 pgs 1-8; Hilkens J., et al, *Journal of*

Biological Chemistry vol **267** pgs 6171-6177). The exact site of cleavage may vary depending on cell type or in response to a disease. The enzyme that cleaves MUC1 may be a membrane-associated enzyme, since the clustered IBRs of the MUC1 receptor limit access to the cleavage sites.

5   One aspect of the invention is the identification of compounds that directly bind to the PSMGFR portion of the receptor. Therefore, a sensitive method for diagnosing early tumors is to administer to the patient, compounds that bind to the PSMGFR region that have also been derivatized with contrast or imaging agents. These compounds will agglomerate onto tumors wherein this portion of the MUC1 receptor is accessible.

10   Compounds described herein that bind to the PSMGFR region as well as other compounds that can be identified using methods of the invention can be readily modified to carry imaging agents. Such imaging agents may include but are not limited to, technetium, rhenium, ^{123}I, and other contrast agents or radioactive entities commonly used in imaging techniques. Imaging techniques include but are not limited to single

15   photon computed tomography (SPECT), MRI, microscopy and the like. In some applications, an attached colloid can act as an imaging agent. Since the carrier for the imaging agent can also be a therapeutic, this technique can combine an early diagnostic with a directed therapeutic.

       As referred to previosly, one aspect of the invention is directed to methods for

20   treating a subject diagnosed with or at risk of developing a cancer or tumor characterized by the aberrant expression of MUC1. The treatments of the present invention involve the use of drugs or "agents" as described herein. That is, one aspect involves a series of compositions useful for treatment of cancer or tumor characterized by the aberrant expression of MUC1, including these compositions packaged in kits including

25   instructions for use of the composition for the treatment of such conditions. That is, the kit can include a description of use of the composition for participation in any biological or chemical mechanism disclosed herein that is associated with cancer or tumor. The kit also can include instructions for use of a combination of two or more compositions of some embodiments of the invention. Instructions also may be provided for administering

30   the drug orally, intravenously, or via another known route of drug delivery. These and other embodiments of the invention can also involve promotion of the treatment of cancer or tumor according to any of the techniques and compositions and combinations of compositions described herein.

In one set of embodiments, patients can be treated with compositions of the invention even though the patients exhibit indication for treatment of one of the compositions of the invention for a condition different from cancer or tumor, including conditions that can be unrelated to cell proliferation or conditions that can accompany

5   cell proliferation, cancer, or tumor. That is, if a composition of the invention is known for treatment of a different condition, some embodiments of the present invention also involve use of that composition for treatments that accompany cell proliferation, cancer, or tumor disease where indicated. These and other embodiments of the invention can include such treatment where the dosage, delivery technique or vehicle, combination

10 with other pharmaceutical compositions or lack of combination with other pharmaceutical compositions, rate of administration, timing of administration, or other factor differs from the use of the composition for treatment of the condition different from cell proliferation, cancer, or tumor. In another set of embodiments, treatment of cell proliferation, cancer, or tumor with compositions of the invention may occur under

15 conditions that are similar to or overlap the use of compositions of the invention for treatment of a different condition, but the compositions of the invention are promoted for treatments that accompany cell proliferation, cancer, or tumor or includes instructions for treatments that accompany cell proliferation, cancer, or tumor as mentioned above. As used herein, "promoted" includes all methods of doing business including methods of

20 education, hospital and other clinical instruction, pharmaceutical industry activity including pharmaceutical sales, and any advertising or other promotional activity including written, oral, and electronic communication of any form, associated with compositions of the invention in connection with treatments that accompany cell proliferation, cancer, or tumor. "Instructions" can and often do define a component of

25 promotion, and typically involve written instructions on or associated with packaging of compositions of the invention. Instructions also can include any oral or electronic instructions provided in any manner. The "kit" typically, and preferably, defines a package including both any one or a combination of the compositions of the invention and the instructions, but can also include the composition of the invention and

30 instructions of any form that are provided in connection with the composition in a manner such that a clinical professional will clearly recognize that the instructions are to be associated with the specific composition.

Subjects for whom certain treatment methods of the invention (with specific compositions directed toward cell proliferation, cancer, or tumor) are not intended are those who are diagnosed with a condition which may already call for treatment with the specific composition. Accordingly, one aspect of the invention involves treatment of cell

5   proliferation, cancer, or tumor with a specific composition disclosed herein for that purpose, not in combination with another agent where the other agent has been taught previously for use in treatment of cell proliferation, cancer, or tumor itself. Another embodiment involves treatment of cell proliferation, cancer, or tumor with this specific composition alone, not in combination with any other active agent. Another embodiment

10   involves treatment of cell proliferation, cancer, or tumor with this specific composition where the use of the composition in the treatment is specifically instructed (through, e.g. written instructions that can accompany the composition) for the treatment of cell proliferation, cancer, or tumor. In a preferred embodiment of this aspect, the invention involves treatment of cell proliferation, cancer, or tumor with the specific composition

15   where the use of the composition in the treatment is specifically instructed to affect a mechanism associated with cell proliferation, cancer, or tumor as disclosed herein.

In yet another set of embodiments, the drugs and agents of the invention can be used for the purpose of disease prevention. In this context, the invention is particularly directed to a patient population never before treated with drugs useful according to

20   certain methods of the invention, including patients who are not suffering from cell proliferation, cancer, or tumor and who may or may not be presently indicating susceptibility to cell proliferation, cancer, or tumor. In other words, the preventative treatment preferably is directed to patient populations that otherwise are free of disease symptoms that call for active treatment with any of the drugs described herein as useful

25   according to the invention.

In one aspect, the invention involves the discovery that calcimycin, fusaric acid, L-α-methyl-dopa, butylindazone, NS1619 and etomoxir interrupt the interaction of MGFR with its ligand(s) that otherwise would bind to MGFR and promote tumorigensis. In this aspect, the invention involves treatment of subjects associated with tumor or

30   cancer associated with aberrant expression of MUC1 with these agents or a combination. These compounds were identified when a drug library was screened using the *in vitro* color change colloid aggregation assay described in Example 5a. These compounds

were then tested in a whole cell assay to determine if they produced the desired activity, namely if they inhibited cell proliferation by interfering with the MGFR-ligand interaction. All of the compounds inhibited cell proliferation, but roughly half of the compounds were toxic to both tumor cells that presented the MUC1 receptor as well as

5   cells that did not present this receptor. As discussed herein, the drug screen described in Example 5a does not differentiate among drugs that inhibit cell proliferation by: (a)binding to or otherwise blocking the activity of a MUC1-associated activating ligand(s), such as growth factors; b) directly binding to the MGFR portion of the MUC1 receptor and blocking its interaction with its activating ligands; or (c) inhibiting the

10   activity of enzymes that modify the MGFR portion of the Muc1 receptor. Drugs that act according to the mode of action described in (a) will not be selective for MUC1-presenting cells and are likely to be somewhat cytotoxic since they inhibit essential growth factors. Drugs that act according to (b) and (c) will selectively inhibit the proliferation of MUC1-presenting cells and further, those that directly bind to the MGFR

15   portion will have little or no toxic effects. Fusaric acid, L-α-methyl-dopa and etomoxir selectively inhibited proliferation of tumor cells presenting MUC1 while leaving control cells unaffected, see Fig. 13.

In one embodiment, the subject to be treated with the above agents can be otherwise free of signs, symptoms or evidence of disorders for which the agents of the

20   invention would normally be or have previously been described. Preferably, the subject is otherwise free of symptoms calling for treatment involving the use of at least any one of calcimycin, fusaric acid, L-α-methyl-dopa, butylindazone, NS1619 and etomoxir, alone or in combination with each other or with other pharmaceutically acceptable substances. For example, calcimycin, fusaric acid, L-α-methyl-dopa, butylindazone,

25   NS1619 and etomoxir may have been suggested for treatment of subjects having certain diseases; thus, in one embodiment, the preferred subjects are free of those disease for which the agents of the present invention have been previously prescribed.

Fusaric acid. Subjects for whom the methods of the invention involving treatment with fusaric acid are not intended are those diagnosed with diseases which

30   already call for treatment with fusaric acid, but where the call for treatment with fusaric acid did not specifically call for treatment directed toward tumors or cancers associated with the abherrant expression of MUC1, particularly in the dosages or other specific

protocols described previously in U.S. Patent No. 6,127,393. Specific diseases listed in U.S. Patent No. 6,127,393 include skin cancer, breast cancer, prostate cancer, cervical cancer, colon cancer, liver cancer and lung cancer. In one embodiment, the methods of the present invention involve treatment with fusaric acid in dosages lower than that

5   described in U.S. Patent No. 6,127,393, as evidenced by graphs of FIG. 13 which are analogous to FIGs. 3A-3C in U.S. Patent No. 6,127,393 which depict an amount of fusaric acid needed to inhibit cell growth.

In one embodiment, the invention provides a fusaric acid treatment in a lower dosage to provide a less than daily administration regimen. For example, the fusaric acid

10 can be provided every other day or once weekly.

Etomoxir. Subjects for whom the methods of the invention involving treatment with etomxir are not intended are those diagnosed with diseases which already call for treatment with etomoxir, particularly those subjects who have diseases associated with chronic heart failure calling for treatment with etomoxir. Such diseases include failing

15 cardiac hypertrophy associated with an inadequate sarcoplasmic reticulum function.

NS1619. Although NS1619 is currently known as a biochemical tool as a K(ca) channel activator, subjects for whom the methods of the invention involving treatment with NS1619 are not intended are those diagnosed with diseases which already call for treatment with NS1619 requiring K(ca) channel modulation.

20 *Calcimycin.* Calcimycin is an ionophorous, polyether antibiotic from streptomyces chartreusensis. Calcimycin binds and transports cations across membranes and uncouples oxidative phosphorylation while inhibiting atpase of rat liver mitochondria. The substance is used mostly as a biochemical tool to study the role of divalent cations in various biological systems. Subjects for whom the methods of the

25 invention involving treatment with calcimycin are not intended are those diagnosed with diseases which already call for treatment with calcimycin in this function.

Butylindazone. R(+)-butylindazone is a KCl cotransport inhibitor, and subjects for whom the methods of the invention involving treatment with butylindazone, specifically R(+)-butylindazone, are not intended are those diagnosed with diseases

30 which already call for treatment with butylindazone requiring inhibition of KCl cotransport.

The method comprises administering to the subject calcimycin, fusaric acid, L-α-methyl-dopa, butylindazone, NS1619 and etomoxir in an amount effective to provide a medically desirable result. In one embodiment, the method comprises administering to the subject any one of calcimycin, fusaric acid, L-α-methyl-dopa, butylindazone,

5   NS1619 and etomoxir in an amount effective to lower the risk/prevent/reduce/inhibit tumors or cancer associated with aberrant expression of MUC1.

The effective amount will vary with the particular condition being treated, the age and physical condition of the subject being treated, the severity of the condition, the duration of the treatment, the nature of the concurrent therapy (if any), the specific route

10   of administration and like factors within the knowledge and expertise of the health practitioner. For example, in connection with tumor or cancer associated with abherrant expression of MUC1, an effective amount is that amount which prevents interaction of MGFR with its ligand that otherwise would promote cell proliferation (for agents that act according to that mechanism, including calcimycin, fusaric acid, L-α-methyl-dopa,

15   butylindazone, NS1619 and etomoxir).

According to alternate mechanisms of drug activity, an effective amount is that amount which maintains self-aggregation of MUC1 receptors (for agents such as polymers or dendrimers that act according to that mechanism). Alternatively, an effective amount is one which reduces levels of cleaved MUC1 IBRs, or maintains low

20   levels of cleaved MUC1 IBRs (for agents that act according to that mechanism). Likewise, an effective amount for treatment would be an amount sufficient to lessen or inhibit altogether the levels of cleaved MUC1 IBR (for agents that act according to that mechanism) so as to slow or halt the development of or the progression of tumor or cancer associated with aberrant expression of MUC1. It is preferred generally that a

25   maximum dose be used, that is, the highest safe dose according to sound medical judgment

When used therapeutically, the agents of the invention are administered in therapeutically effective amounts. In general, a therapeutically effective amount means that amount necessary to delay the onset of, inhibit the progression of, or halt altogether

30   the particular condition being treated. Generally, a therapeutically effective amount will vary with the subject's age, condition, and sex, as well as the nature and extent of the disease in the subject, all of which can be determined by one of ordinary skill in the art.

poly(lauryl methacrylate), poly(phenyl methacrylate), poly(methyl acrylate),

poly(isopropyl acrylate), poly(isobutyl acrylate), and poly(octadecyl acrylate). Thus, the

invention provides a composition of the above-described agents for use as a medicament,

methods for preparing the medicament and methods for the sustained release of the

5   medicament in vivo.

The compositions may conveniently be presented in unit dosage form and may be

prepared by any of the methods well known in the art of pharmacy. All methods include

the step of bringing the therapeutic agents into association with a carrier which

constitutes one or more accessory ingredients. In general, the compositions are prepared

10   by uniformly and intimately bringing the therapeutic agent into association with a liquid

carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product.

Compositions suitable for parenteral administration conveniently comprise a

sterile aqueous preparation of the therapeutic agent, which is preferably isotonic with the

blood of the recipient. This aqueous preparation may be formulated according to known

15   methods using those suitable dispersing or wetting agents and suspending agents. The

sterile injectable preparation may also be a sterile injectable solution or suspension in a

non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,

3-butane diol. Among the acceptable vehicles and solvents that may be employed are

water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed

20   oils are conventionally employed as a solvent or suspending medium. For this purpose

any bland fixed oil may be employed including synthetic mono or di-glycerides. In

addition, fatty acids such as oleic acid find use in the preparation of injectables. Carrier

formulations suitable for oral, subcutaneous, intravenous, intramuscular, etc. can be

found in Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA.

25   Compositions suitable for oral administration may be presented as discrete units

such as capsules, cachets, tablets, or lozenges, each containing a predetermined amount

of the therapeutic agent. Other compositions include suspensions in aqueous liquors or

non-aqueous liquids such as a syrup, an elixir, or an emulsion.

Other delivery systems can include time-release, delayed release or sustained

30   release delivery systems. Such systems can avoid repeated administrations of the

therapeutic agent of the invention, increasing convenience to the subject and the

physician. Many types of release delivery systems are available and known to those of

ordinary skill in the art. They include polymer based systems such as polylactic and

polyglycolic acid, poly(lactide-glycolide), copolyoxalates, polyanhydrides, polyesteramides, polyorthoesters, polyhydroxybutyric acid, and polycaprolactone. Microcapsules of the foregoing polymers containing drugs are described in, for example, U.S. Pat. No. 5,075,109. Nonpolymer systems that are lipids including sterols such as

5 cholesterol, cholesterol esters and fatty acids or neutral fats such as mono-, di- and tri-glycerides; liposomes; phospholipids; hydrogel release systems; silastic systems; peptide based systems; wax coatings, compressed tablets using conventional binders and excipients, partially fused implants and the like. Specific examples include, but are not limited to: (a) erosional systems in which the polysaccharide is contained in a form

10 within a matrix, found in U.S. Patent Nos. 4,452,775, 4,675,189, and 5,736,152, and (b) diffusional systems in which an active component permeates at a controlled rate from a polymer such as described in U.S. Patent Nos. 3,854,480, 5,133,974 and 5,407,686. In addition, pump-based hardware delivery systems can be used, some of which are adapted for implantation.

15   Use of a long-term sustained release implant may be particularly suitable for treatment of established cancer conditions as well as subjects at risk of developing a cancer. "Long-term" release, as used herein, means that the implant is constructed and arranged to deliver therapeutic levels of the active ingredient for at least 7 days, and preferably 30-60 days. The implant may be positioned at the site of the tumor.

20 Long-term sustained release implants are well known to those of ordinary skill in the art and include some of the release systems described above.

The therapeutic agent may be administered in alone or in combination with an anti-cancer drug. If the therapeutic agent is administered in combination the compounds may be administered by the same method, e.g. intravenous, oral, etc. or may be

25 administered separately by different modes, e.g. therapeutic agent administered orally, anti-cancer drug administered intravenously, etc. In one embodiment of the invention the therapeutic agent and the anti-cancer drug are co-administered intravenously. In another embodiment the therapeutic agent and the anti-cancer drug are administered separately.

30   Anti-cancer drugs that can be co-administered with the compounds of the invention include, but are not limited to Acivicin; Aclarubicin; Acodazole Hydrochloride; Acronine; Adriamycin; Adozelesin; Aldesleukin; Altretamine; Ambomycin; Ametantrone Acetate; Aminoglutethimide; Amsacrine; Anastrozole;

Example 2: Dimerization of the MGFR portion of the MUC1 receptor triggers enhanced
Cell Proliferation Consistent with the Mechanism Presented for MUC1 Tumor Cells

This example demonstrates the effect of dimerization on the MUC1 receptor. In

this example it is shown that exposure of cells to a bivalent antibody grown against the

5   MGFR region of the MUC1 receptor, at varying concentration, results in enhanced cell

proliferation (or lack thereof) consistent with the mechanism presented for MUC1 tumor

cells. A bivalent antibody was raised against PSMGFR (i.e., a single antibody having

the ability to bind simultaneously to two MGFRs was produced). MUC1 tumor cells

(T47Ds) were exposed to this antibody, and cell proliferation was studied as a function

10   of concentration of the antibody. A growth/response curve typical of a growth

factor/receptor - antibody response was observed. Specifically, at concentration low

enough that only a small portion of the cells were exposed to the antibody, cell

proliferation was low. At a concentration of antibody high enough that one antibody

could bind adjacent MGFRs, cell proliferation was maximized. At a high excess of

15   antibody, each antibody bound only a single MGFR, rather than dimerizing adjacent

MGFRs, and proliferation was reduced.

T47D (HTB-133) cells, a human breast cancer cell line that overexpresses

MUC1, were cultured to 30% confluency. An antibody raised against the PSMGFR

portion of the MUC1 receptor, i.e. an antibody to the MFGR (Zymed, San Francisco,

20   California, USA), was added to cells at varying concentrations in a multi-well cell

culture plate. As a negative control, a second set of T47D cells was treated with an

irrelevant antibody (anti-streptavidin). Prior to adding antibody, cells were counted (at

time zero). All experiments were performed in triplicate. Cells were allowed to grow in

a CO_2 incubator under normal conditions. Cells were counted using a hemacytometer (3

25   counts per well) at 24 hours and again at 48 hours. Results, see Fig. 4, show that in a

concentration-dependent manner, addition of antibody caused enhanced cell proliferation

compared to the proliferation of the same cells treated with a control antibody. Figure 4

is a graph in which measured cell growth at 24 and 48 hours is plotted as a function of

anti-PSMGFR concentration. At the optimal antibody concentration, when presumably

30   one antibody binds bivalently to two MGFR portions of the MUC1 receptor, i.e.

dimerizes the receptor, cell proliferation is at a maximum.

In a similar experiment, a concentration of the anti-PSMGFR antibody,

identified to maximize cell proliferation, was added to a first group of T47D tumor cells,

grown as described above. The same amount of the anti-PSMGFR antibody was added to a set of control cells, K293 cells. Figure 5 shows that the addition of the anti-PSMGFR antibody to MUC1 tumor cells (T47D) enhanced proliferation by 180% 24 hours, but had no effect on the control cells. The growth of the T47D cells plateaued to saturation, for cells with added antibody, at 48 hours. Control cells never reached saturation within the time frame of the experiment and were at 70% confluency at 48 hours.

Example 3a: The Activating Ligand Produced by MUC1-Presenting Cells Binds Multiple PSMGFRs

        In this example, it is demonstrated that the activating ligand that triggers MUC1 tumor cell proliferation binds multiple PSMGFRs simultaneously. Colloid particles were produced that carry immobilized PSMGFRs, and suspensions of these colloids were exposed to lysate and supernatants of (1) MUC1 tumor cells, or (2) control cells. MUC1 tumor cell lysates/supernatants caused the colloids to aggregate (suspension turns blue) because the activating ligand contained in them binds MGFRs on different colloid particle, causing the colloid particles to aggregate. The control cell lysates/supernatants do not.

        20 microliters of a 100 micro molar solution of the His-PSMGFR peptide, described in Table 1, were added to 100 microliters of colloids which were derivatized with a SAM including NTA-Nickel moieties to capture the histidine-tagged peptides. Lysates and supernatants from four different tumor-associated cell lines (HTB-133 (also called T47D), CRL-1500, CRL 1504 and CRL-1902; ATTC, American Type Culture Collection, Manasses, VA) were added to aliquots of the peptide-presenting colloids. To each well of a 96-well plate, 30 uL of each lysate/supernatant was added to 40uL of PBS, 30ul of the His-PSMGFR-bearing or as a negative control, the GST-bearing colloids. A color change from pink to blue rapidly occurred for the two cell lines that overexpress the MUC1 receptor. A color change was observed for the CRL-1504 cells, which express MUC1, but after a much longer incubation period. No color change occurred for the cell line CRL-1902, which are not known to express the MUC1 receptor. As a negative control, the lysates were also added to colloids presenting an irrelevant peptide, the GST protein. As an additional negative control (data not shown), growth media for

the different cell lines was added to colloid preparations. No color change was observed for the negative controls.

Results are shown in Figure 6, which is an image of a section of a 96-well plate illustrating the colloid-based color change assay.

5   Referring still to Figure 6, wells in Column 1 contain colloids bearing His-PSMGFR. Wells in Column 2 contain colloids bearing GST (glutathione-S-transferase) protein as a negative control. Row B: HTB-133 (also known as T47D) color change from pink to blue; Row C: CRL-1500 color change from pink to blue; Row A: CRL-1504 color change from pink to blue, but after a much longer incubation period; Row D: 10   CRL-1902 no color change was produced. As a negative control, the lysate/supernatants were also added to colloids presenting an irrelevant peptide, the GST protein, but no color change was observed for any cell line.

The results of Fig. 7 argue that this is a specific interaction between the PSMGFR peptide and a ligand(s) rather than random precipitation of colloids. Fig. 7 is an image of 15   96-well plate illustrating a colloid-based color-change binding assay between the His-PSMGFR, MUC1-derived peptide and a ligand(s) present in a crude cell lysate. The addition of imidazole, which releases the histidine-tagged probe peptide from the NTA moiety on the colloid, caused a reversal of the color change, which argues that the color change is the result of a specific interaction rather than random colloid aggregation. 20   Addition of a ligand present in a crude cell lysate of T47D cells, which may be a dimer under these conditions, to the peptide-presenting colloid solutions, caused the solution to turn blue, see Figure 7 Row B, (at arrow). A drug candidate that disrupts the peptide-ligand interaction will cause the solution to remain or revert to pink. In this way, high throughput drug screening is achieved and may also be automated by analyzing color 25   change on a spectrophotometer.

These results indicate that cell lines HTB-133 (T47D) and CRL-1500 secrete high levels of the ligand for the MUC1 receptor and that the ligand acts to dimerize or multimerize the receptor.

30   Example 3b: Relationship Between Degree of Expression of MUC1 in Cell Lines and
Presence of Ligand That Interacts With PSMGFR

In this example, it was shown that there is a direct correlation between the expression of MUC1 and the presence of a ligand or ligands that binds to a peptide

derived from the portion of the MUC1 receptor that is directly adjacent to the cell surface (MGFR).

Colloid particles carrying immobilized PSMGFR (His-PSMGFR linked to NTA-presenting thiols in SAMs on the colloids) were observed for their aggregation potential
5   (color change from pink to blue in suspension) upon exposure to lysates from various cells lines know to overexpress, express, or not express MUC1. HTB-133, CRL-1500, CRL-1504, and CRL-1902 lines were studied. Lysates from a cell line that overexpresses MUC1 (HTB-133) caused colloid suspensions to turn blue within 15 minutes, indicating a high concentration of a ligand(s) in the lysate that interacts with the
10 colloid-immobilized MGFR-derived peptides. Lysates from cell lines that express, but do not overexpresses, MUC1 (CRL-1500 and CRL-1504) caused colloid suspensions to turn blue within 3 hours, indicating moderate concentration of ligand(s) in the lysate. Lysates from a cell line that is not known to express MUC 1 (CRL-1902) caused colloid suspensions to begin to turn blue only after 10 hours, indicating a low concentration of a
15 ligand(s) in the lysate. Controls involved immobilized RGD peptide (which is not dimerized by components of these cell lysates). The control suspensions remained pink indefinitely, indicating no aggregation.

See Figs. 8A-15D. Rows A-D contained colloid particles carrying immobilized His-PSMGFR. Rows E-H contained colloid particles carrying a random sequence
20 peptide. Columns 2, 5, 8, and 11 contained lysates from a tumor cell line that overexpresses MUC1 (HTB-133). Columns 3, 6, 9, and 12 contained lysates from a tumor cell line that does not expresses MUC1 (CRL-1902). Columns 1, 4, 7, and 10 contain lysates from a tumor cell line that expresses, but does not overexpress, MUC1 (CRL-1504). Columns 1-3: NTA concentration on colloid: 20 micromolar; columns 4-
25 6: 40 micromolar; columns 7-9: 60 micromolar; columns 10-12: 80 micromolar, all in total thiol concentration of 600 micromolar in deposition solution. Fig. 8A: time = 0; Fig. 8B: time = 15 minutes; Fig. 8C: time = 1 hour; Fig. 8D: time = 3 hours.

Overall, these results point to a mechanism involving a feedback loop involving both ligand production and aberrant expression of the MUC1 receptor.
30

Example 4a: Identification of Ligands that bind to the MGFR portion of the MUC1 receptor

      In an effort to identify ligands to the MUC1 receptor, synthetic, His-PSMGFR peptides,

5   GTINVHDVETQFNQYKTEAASPYNLTISDVSVSDVPFPFSAQSGAHHHHHH (SEQ ID NO: 2), which represents the portion of the MUC1 receptor, that remains attached to the cell surface after cleavage of the interchain binding region, were loaded onto NTA-Ni beads (cat. #1000630; available from Qiagen GmbH, Germany) and incubated with cell lysates in the presence (Fig. 9) or absence (Fig. 10) of the protease inhibitor PMSF

10   (phenyl methyl sulfonyl fluoride). Lysates from T47D cells were used because this breast tumor cell line was known to overexpress MUC1; additionally, the inventors presented evidence herein (see Fig. 8A-D) that this cell line also overexpresses MUC1 ligand(s). T47D cells were cultured then sonicated for 1 minute to lyse the cells. Lysates were mixed with the PSMGFR peptide-presenting beads and incubated on ice with

15   intermittent mixing for 1hr. As a negative control, an irrelevant peptide, HHHHHHRGEFTGTYITAVT, was attached to NTA-Ni beads and treated identically. Both sets of beads were washed 2X with phosphate buffer pH 7.4. Bound protein species were eluted by 3 additions of 100uL of phosphate buffer that also contained 250mM imidazole. For both the peptides, a portion of the first elution was removed and

20   reserved to run as a separate sample, while the remainder was combined with the other 2 elutions and concentrated by TCA (tri-chloro acetic acid)-precipitation (Chen, L. et al., Anal. Biochem. Vol 269; pgs 179-188; 1999). Eluates were run on a 12% SDS gel, see Figure 9. The gel was then silver stained (Schevchenko, A et al; Anal. Chem., Vol. 68; pg 850-858; 1996). Lanes were loaded as follows: (from left to right) (1) Benchmark

25   pre-stained protein ladder (Gibco); (2) first eluate from the MUC1 peptide; (3) 1/10th of TCA-concentrated sample; (4) blank; (5) 9/10th TCA- concentrated sample; (6) first eluate negative control peptide; (7) 1/10th of TCA-concentrated sample from the negative control peptide; (8) 0.5 picomoles BSA (as a standard); (9) 9/10th TCA- concentrated sample from the negative control peptide; (10) silver stain SDS page standard (BioRad

30   cat. #1610314). Referring now to Fig. 9, comparing lanes 2 and 6 (control), it can be seen that the MUC1 PSMGFR peptide bound distinguishably to three peptides: a first unique peptide that runs at an apparent molecular weight of 17kD; and a second peptide

(more intense band) that runs at an apparent molecular weight of 23kD. Note that in lane 5, where the sample is the most concentrated, a third unique band is seen at about 35kD.

Figure 10 shows the results of an experiment, which was identical to that shown in Fig. 9, with the exception that the protease inhibitor PMSF was not added. PMSF

5 binds to and blocks the action of several enzymes, such as proteases. This experiment was performed, in the absence of PMSF, to determine whether an enzyme present in the lysate was a ligand of the MUC1 receptor. Referring now to Fig. 10, comparing lanes 3 (control) and 7, it can be seen that the MUC1, PSMGFR peptide bound distinguishably to one peptide, with an apparent molecular weight of 35kD. Note that this band was

10 visible in Fig. 9 (with PMSF), but was much fainter and only co-eluted from the most concentrated sample. These results are consistent with the idea that the PFMGFR portion of the MUC1 receptor is a substrate for a ligand of apparent molecular weight of about 35kD and which may bean enzyme. As mentioned elsewhere herein, drug screens based on inhibition of binding between the PSMGFR and this ligand or the ligand in a

15 crude cell lysate can identify compounds that inhibit the action of this enzyme.

Table 2. Cell lines were purchased from the ATCC (American Type Culture Collection, Manasses, VA) and are all breast carcinoma cell lines. Some lines have been shown to express or over express the tumor marker receptor MUC1, Her2/neu or the oncogenic

20 enzyme cathepsin K.

Cell line	Gel Result Co-elutes with PSMGFR peptide	Color change assay – yes, turned blue	Expression of species in cell line	Common name	ATCC name
1.	+++	++++	Expresses MUC1	T-47D	HTB-133
2.	+	-	ND on MUC1 over expresses HER2/neu	UACC-893	CRL-1902
3.	+++	++++	Overexpresses MUC1	ZR-75-1	CRL-1500
4.	++	+	Express MUC1 over express cathepsin K	ZR-75-30	CRL-1504

Table 3

Cell line	Growth Media
HTB-133	RPMI 1640 media, purchased from Mediatech supplemented with 1 mM sodium pyruvate, 10% FBS, 4.5 g/L glucose and 1.5 g/L sodium bicarbonate, with 2 IU bovine insulin per mL.
CRL-1902	Liebovitz L-15 media (Sigma), supplemented with 10% FBS
CRL-1500	RPMI 1640 media from Mediatech supplemented with 1 mM sodium pyruvate, 10% FBS, 4.5 g/L glucose and 1.5 g/L sodium bicarbonate
CRL-1504	RPMI 1640 media from Mediatech supplemented with 1 mM sodium pyruvate, 10% FBS

Example 4b: Demonstration that the Ligand That Interacts with MUC1 Cancer Cells is a Multimer

In this example, it is demonstrated that a ligand produced by MUC1 cancer cells that triggers cell proliferation in these cells is a multimer.

Protein bands at 17 kD, 23 kD, and 35 kD were excised from the gels described above in Example 4a and submitted for peptide analysis. These gel bands purportedly

contained ligands to the MGFR region of the MUC1 receptor. Recall that the 17 kD and 23 kD species bound to the MGFR peptide in the presence of the protease inhibitor, PMSF, while the 35 kD species bound when PMSF was not added to the cell lysate mixture.

The following peptide analysis was performed. Samples derived from the gel slices were proteolytically digested. Fragments were then separated by microcapillary HPLC which was directly coupled to a nano-electrospray ionization source of an ion trap mass spectrometer. MS/MS spectra was obtained on-line. These fragmentation spectra were then correlated to known sequences using the SEQUEST® algorithm in conjunction with other algorithms. Results were then manually reviewed to confirm consensus with sequences of known proteins.

Peptide sequences contained within both the 17 kD and the 23 kD bands (PMSF added to lysate) corresponded to a protein known as Metastasis Inhibition Factor NM23, which has been implicated in both the promotion and inhibition of metastasis of human cancers. Whether the role of NM23 is a tumor supressor or promoter may depend on the type of cancer. In ovarian, colon and neuroblastoma tumors, NM23 overexpression has been linked to a more malignant phenotype (Schneider J, Romero H, Ruiz R, Centeno MM, Rodriguez-Escudero FJ, "NM23 expression in advanced and borderline ovarian carcinoma", *Anticancer Res*, 1996; 16(3A): 1197-202). However, breast cancer studies indicate that reduced expression of NM23 correlates with poor prognosis (Mao H, Liu H, Fu X, Fang Z, Abrams J, Worsham MJ, "Loss of nm23 expression predicts distal metastases and poorer survival for breast cancer", Int J Oncol 2001 Mar;18(3):587-91).

The sequences that were identified from the protein gel band described in Figures 9 and 10 and that are derived from a protein implicated in many cancers called Metastasis Inhibition Factor NM23 are shown below in Table 4. NM23 exists as a hexamer and may recognize an unmodified form of the MGFR portion of the MUC1 receptor.

Peptide sequences that were identified from the 35 kD gel band (PMSF NOT added to lysate) corresponded to more than one protein species, including 14-3-3, which is a signaling protein implicated in many cancers, and cathepsin D, which is a protease and is also implicated in tumor progression. 14-3-3 exists as a dimer and can simultaneously bind to two, identical phospho-serine peptides. This would dimerize the MGFR portion of the MUC1 receptor to trigger cell proliferation, which is consistent

with the mechanism presented herein. Cathepsin D is a protease and may be involved in the cleavage of the MUC1 receptor.

The identity of these ligands is consistent with the MUC1-dependent cell proliferation mechanism that is disclosed herein, i.e., a ligand that dimerizes the MGFR
5   portion of the MUC1 receptor triggers cell proliferation and cleavage of only a portion of the MUC1 extracellular domain exposes the functional part of the receptor which is defined by most or all of the PSMGFR sequence given in Table 1.

Consistent with methods of the invention, a therapeutic strategy is to identify compounds that either interrupt the interaction of one of the ligands with the MGFR
10   portion of the MUC1 receptor, or to identify compounds that bind to and block the action of the ligand(s).

Table 4

17 kD species identified herein from gel band
15   1) Metastasis Inhibition Factor NM23   gi: 127982
TFIAIKPDGVQR
VM*LGETNPADSKPGTIR
VMLGETNPADSKPGTIR
NIIHGSDSVK
20   GLVGEIIKR
GLVGEIIK

23 kD species identified herein from gel band
1) Metastasis Inhibition Factor NM23   gi: 127982
25   TFIAIKPDGVQR
YM*HSGPVVAM*VWEGLNVVK

35 kD identified herein from gel band
1) 14-3-3 epsilon   gi: 5803225
30   AAFDDAIAELDTLSEESYK
AASDIAM*TELPPTHPIR
YLAEFATGNDR
DSTLIMQLLR

YDEMVESMK

VAGM*DVELTVEER

HLIPAANTGESK

5   2) cathepsin D   gi:4503143

DPDAQPGGELM*LGGTDSK

DPDAQPGGELMLGGTDSK

ISVNNVLPVFDNLM*QQK

ISVNNVLPVFDNLMQQK

10   QPGITFIAAK

3) human annexin V with Proline substitution by Thrionine gi: 3212603

GLGTDEESILTLLTSR

DLLDDLKSELTGK

15   SEIDLFNIR

Examples 5a-d: Drug Studies Consistent with Mechanism Presented for MUC1 Cancer

In these examples, drugs that inhibit proliferation in MUC1 tumor cells
specifically were compared to drugs that inhibit proliferation in both MUC1 tumor cells
20   and other cells. Drugs, both specific and non-specific, were identified by exposing them
to PSMGFR-presenting colloids in the presence of MUC1 tumor cell lysates. Drugs
were identified as those that prevented colloid-colloid interactions. Cell studies resulted
in a separation of these drugs into two groups - a group specific for MUC1 tumor cells
and a non-specific group. Non-specific drugs did not bind to PSMGFR, but are
25   presumed to bind the activating ligand, and inhibit proliferation of control cells as well
as MUC1-presenting cells. Additionally, this group of drugs was somewhat toxic to both
cell types, since they remove the activating ligand from interaction with the cells. Drugs
specific for MUC1 tumor cells were found to bind to PSMGFR on beads, as
demonstrated by HPLC analysis of the product of cleavage of PSMGFR from the beads.

30

Example 5a: Drug Screening Assay Using Colloid-Based Colorimetric Detection to Identify Agents that block the Interaction of the MGFR portion of the MUC1 Receptor with its Activating Ligand(s)

The following is an example of a working drug screening assay to identify anti-cancer agents. In this example, a histidine-tagged peptide derived from the portion of the MUC1 receptor that remains attached to the cell surface after receptor cleavage (His-PSMGFR) was attached to NTA-nickel-SAM-coated gold colloids. The peptide-presenting colloids were incubated with lysates/supernatants from MUC1 presenting cells that were shown herein to contain ligands that cause dimerization or multimerization of that portion of the MUC1 receptor. This ligand-induced multimerization of the MGFR portion of the receptor causes the attached colloids to be drawn close together, which causes a change in the color of the colloid solution from pink to blue. Drugs that interfere with the binding of activating ligands to the MGFR portion of the receptor cause the solution to remain pink.

NTA-SAM-coated colloids presenting the PSMGFR peptide were incubated with cell lysates/supernatants from T47D cells, which we previously showed by gel-electrophoresis to contain the ligand to MUC1 (Example 4a). Negative control wells contained colloids bound with a random sequence histidine-tagged peptide in place of the MUC1 peptide. Figure 11, which is an image of 96-well plate illustrating a color-change binding assay, shows the results of the experiment. The well containing colloids that presented the PSMGFR peptide plus T47D cell lysates (Well A) changed color from pink to blue, indicating the presence of a multimerizing ligand. Wells that contained a random sequence peptide (RGD) in place of the PSMGFR peptide (Well B) remained pink. Wells that contained phosphate buffer in place of cell lysate also remained pink (Wells C and D).

The data below demonstrates the ability of anti-tumor drugs identified in accordance with the invention, specifically, calcimycin, fusaric acid, L-α-methyl-dopa, butylindazone, NS1619 and etomoxir to inhibit proliferation of cells that aberrantly express MUC1, by blocking the interaction of MGFR with ligands that promote cell proliferation.

An experiment similar to that described above was run in the presence of drug candidates to determine whether candidates could be identified that interfere with ligand/MGFR binding.

T47D cells were trypsinized from a T25 flask, pelleted, resuspended in phosphate
buffer, and lysed by sonication to release the ligand into solution. NTA-SAM-coated
colloids were bound with the His-PSMGFR peptide:
GTINVHDVETQFNQYKTEAASPYNLTISDVSVSDVPFPFSAQSGAHHHHHH

5   200μl NTA-SAM-coated colloids were incubated with 20μl 100μM peptide in phosphate
buffer for 15 minutes, pelleted to remove unbound peptide, and resuspended in
phosphate buffer. Negative control colloids were incubated with a random sequence
histidine-tagged peptide in place of the MUC1 peptide. The cell lysate (65μl) was
mixed with 5μl drug candidate in DMSO and added to 30μl MUC1-peptide-bound

10   colloids in the wells of an ELISA plate for a final drug concentration of approximately
100μM. Positive controls contained DMSO in place of a drug candidate; negative
controls contained DMSO in place of a drug candidate, and colloids bound with a
random sequence peptide in place of the MUC1 peptide. A color change from pink to
blue indicates that the ligand in the cell lysate bound to the MUC1-peptide, dimerizing

15   the peptide, and bringing the colloids into close enough proximity with one another to
cause a color change. Positive controls, which do not contain a drug candidate, change
color from pink to blue within two hours, as there is nothing to inhibit the interaction
between the MUC1 peptide and the ligand present in the cell lysate. A lack of color
change (wells remain pink) indicates that the drug candidate blocked the interaction

20   between the MUC1 peptide and the cognate ligand, either by binding to the MGFR
portion of the MUC1 receptor, inhibiting a modifying enzyme, or by binding to its
activating ligand. Negative control wells, which contain colloids presenting a random
sequence peptide in place of the MUC1 peptide, remain pink, as the ligand to the MUC1
peptide will not dimerize the random sequence peptide. Figure 12 shows a sample drug-

25   screening plate used in the assay described above. Positive control wells (A1-D1)
changed color from pink to blue within two hours, while negative control wells (E1-H1)
remained pink. Well E6 contains a drug that inhibited the interaction between the MUC1
peptide and the cognate ligand, causing the well to remain pink.

Example 5b1: Secondary Drug Screen to Determine Mode of Action of Identified Drug
Agents - Proliferation of Cells Treated With Drug Candidates That Disrupt the
Interaction of MUC1 With its Natural Ligand

Previously, we showed in vitro that the ligand to the MGFR portion of the MUC1

5   receptor caused dimerization, or multimerization of the PSMGFR peptide on colloids,

see Example 3a. We also showed that dimerization of the MGFR portion of the receptor

induced an enhanced cell proliferation, see Example 2. It then follows that agents that

block the interaction of the MGFR portion of the receptor with its activating ligands will

block the proliferation of MUC1-presenting tumor cells. Therefore, drugs that were

10 identified using the in vitro drug screening assay described in Example 5a were tested in

a functional assay to determine their ability to inhibit MUC1-dependent cell

proliferation.

T47D cells, mammary carcinoma cells known to overexpress MUC1, were grown

in the wells of an ELISA plate along with K293 cells, human embryonic kidney cells

15 which will serve as the negative control. 100µl cells in growth media were added to the

wells of an ELISA plate and the cells were allowed to adhere overnight. The number of

cells in each well on the plate were then counted and recorded. 1µl of a drug candidate

in DMSO was then added to each well of both the T47D cells and the K293 cells. 1µl

DMSO alone was added to control wells. Each well was repeated in triplet. The cells

20 were allowed to grow for 48 hours, the normal doubling time for these cell lines. The

number of cells in each well was again counted and recorded. The percent cell growth

over this 48-hour period was calculated, and the percent cell growth for wells containing

a drug candidate versus DMSO were compared. As seen in Fig. 13, Etomoxir, L-alpha-

methyl DOPA, and Fusaric acid selectively inhibited proliferation of the MUC1-

25 expressing tumor cells over K293 negative control cells. The DMSO control cells (both

T47D and K293) show that DMSO alone does not effect cell proliferation. Fig. 13 is a

histogram illustrating the selective inhibition of proliferation of tumor cells that

aberrantly express the MUC1 receptor (T47D cell line), in response to treatment with

compounds of the invention, and lack of an effect on cells that do not express MUC1

30 (K293). Cell growth in the presence of Etomoxir was -10.2% for T47D cells and 94%

for control cells (K293); Cell growth in the presence of L-alpha-methyl-DOPA was 20%

for T47D cells and 110% for control cells (K293); Cell growth in the presence of

Fusaric acid was 27.7% for T47D cells, and 106.7% for control cells (K293); Cell

growth in the absence of any drug (but with an equivalent amount of DMSO added) was 91.3% for T47D cells, and 106.2% for control cells (K293).

Example 5b2: Drugs Identified with the Colorimetric in vitro Drug Screen Separate into Two Categories – Selective for MUC1-Presenting Cells and Non-Selective

As discussed, compounds identified in the in vitro drug screening assay (described in Example 5a), which identifies compounds that interfere with the interaction between the MGFR portion of the MUC1 receptor and its activating ligands, can inhibit cell proliferation by three modes of action. These drugs can a) block the activity of the activating ligand(s), that act as growth factors; b) directly bind to and block the MGFR portion of the receptor; or c) inhibit the activity of enzymes that modify the MUC1 receptor. It is expected that drugs that function by (a) will inhibit proliferation of a variety of cell types, while those that function according to (b) and (c) will selectively inhibit the proliferation of MUC1-presenting cells.

Figure 14 is an image of a multi-well plate in which the colorimetric drug screening assay (see Example 5a) identified several compounds (each designated by a MN#) that interfered with the interaction of the MGFR portion of the MUC1 receptor and a multimerizing ligand(s). All of the drug-containing wells demonstrate interference with ligand binding as evidenced by each of the wells either remaining pink or turning purple, indicative of binding being essentially eliminated or reduced over positive binding controls (top three wells of right-most column, which are blue). Wells in the top half of the plate (rows A-C) contain drugs that were shown in the functional cell proliferation assay (see Example 5b) to selectively inhibit the proliferation of MUC1-presenting tumor cells by either directly binding to the MGFR portion or by acting on its modifying enzymes. Figure 15 is a bar graph that compares the percentage cell growth of MUC1 tumor cells (T47Ds) to a control cell line (K293s), in response to treatment with novel drugs, (described in greater detail in commonly-owned, co-pending U.S. provisional patent applications serial nos. 60/317,302 and 60/317,314, both filed on September 5, 2001 and entitled COMPOSITIONS AND METHODS OF TREATMENT OF CANCER). As is readily apparent, this group of drugs dramatically inhibited or completely prevented the proliferation of MUC1-presenting tumor cells, while leaving the control cells, in most cases, unaffected.

Wells in the bottom half of the plate (rows D-G) contain drugs that were shown

in the cell proliferation assay to act non-selectively as they inhibited the proliferation of

both cell types. Figure 16 is a bar graph that shows the effect of these novel drugs

(described in greater detail in commonly-owned, co-pending U.S. provisional patent

5   applications serial nos. 60/317,302 and 60/317,314, both filed on September 5, 2001 and

entitled COMPOSITIONS AND METHODS OF TREATMENT OF CANCER) on cell

growth for MUC1-presenting cells (T47D) and a control cell line (K293). Notably, this

group of drugs, which presumably bind to growth factors, is toxic to the cells as one

skilled in the art would expect for agents that act on growth factors.

10

Example 5c: Co-Elution of Drugs With PSMGFR Peptide Proves Direct Binding to MGFR

Herein, we show that several drugs inhibited MUC1-dependent cell proliferation

by binding to the MGFR portion of the MUC1 receptor and disrupting the interaction

15   between the MGFR portion and its cognate ligand. The direct binding of several drugs to

the PSMGFR peptide was demonstrated using two methods. The His-PSMGFR peptide

was attached to NTA-nickel agarose beads then separately incubated with each of the

drug candidates. 100μl beads were bound to saturation with 1mg peptide, rinsed to

remove unbound peptide, and incubated with 25μl 2.7mg/ml drug candidate in DMSO

20   for one hour in 5ml phosphate buffer. Unbound drugs were washed away with

phosphate buffer, and the peptide was eluted from the beads with PBS, 250mM

imidazole. If the drug candidate bound to the peptide, it would co-elute with the peptide

in the imidazole solution. Peptide-drug complexes were then separated by HPLC. HPLC

elution peaks from the complex were compared to the elution peaks from the drug,

25   injected alone, and the PSMGFR peptide alone. In a pilot study, 3 drugs, chosen

randomly from the group of drugs that selectively inhibit MUC1 cell proliferation, were

compared to 3 drugs chosen randomly from the group that non-selectively inhibited cell

proliferation. As seen in Figure 17, the drugs on the left, chosen from the selective

group, bind to the PSMGFR peptide, while drugs on the right, chosen from the non-

30   selective group, did not.

In a similar experiment, eluates from the beads were analyzed by TLC (thin layer

chromatography). Two drugs, calcimycin and NS1619 which could be tracked by TLC,

because they fluoresced under UV light, were tested. NTA-nickel bead-immobilized

peptides were incubated with the drugs as described above, rinsed and eluted from the

beads, then rotoevaporated to remove the aqueous buffer. The solid was then

resuspended in ethylacetate, 5% methanol and spotted on TLC plates then run in the

same organic solvent. Both drugs co-eluted with the PSMGFR peptide, showing

5   conclusively that the drugs bind to the PSMGFR peptide. Calcimycin, gave a clear blue

spot under 250nm UV light and ran at a less polar position (as expected) than the peptide

itself. The second drug, NS1619, gave a less visible spot at a slightly less polar position

than the peptide. The TLC plate was stained with iodine to reveal the peptide spots,

which are visible due to the presence of tyrosine and phenylalanine residues.

10

Prophetic Example 5d: Discriminating between drugs that inhibit MUC1-dependent cell
proliferation by binding to and blocking the MGFR portion of the MUC1 receptor and
those that inihibit selective proliferation by acting on enzymes that modify the MGFR
portion.

15   This experiment is designed to distinguish drugs that selectively inhibit MUC1

cell proliferation by inhibiting modifying enzymes from drugs that inhibit by binding to

and blocking the MGFR portion of the MUC1 receptor. Ex. 8 below shows that

ligand(s) present in the MUC1 lysate were not able to bind PSMGFR peptide when the

enzyme inhibitor PMSF was added to the lysate. This implied that the PSMGFR is first

20   modified before it recognizes its cognate ligand(s). Using the drug screening assay

described in Ex. 5a, one cannot differentiate between drugs that will selectively block the

proliferation of MUC1-presenting cells and those that block the proliferation of a wide

variety of cells, i.e. by inhibiting the MUC1 ligands that act as growth factors. To

identify drugs that are selective for MUC1, drug hits are subjected to a secondary assay,

25   which measures the percentage cell proliferation of MUC1 cells compared to control

cells. Of the drugs that are selective for MUC1-presenting cells, neither assay can

differentiate between drugs that bind to and block the MGFR and those that inhibit its

modifying enzymes. Since enzyme modified peptides migrate through a gel at a slower

rate than unmodified peptides, one can use this difference in gel mobility to determine

30   which drugs act by inhibiting the enzyme(s).

To determine which drugs function via which of these two mechanisms, drugs

are incubated with lysates and supernatants from MUC1-presenting cells, to allow the

drugs to inhibit MUC1-modifying enzymes. The synthetic His-PSMGFR peptide,

immobilized on commercially available NTA-nickel beads, are then mixed with this

lysate mixture. After a 30-minute incubation on ice, excess lysate and drug are discarded

in the supernatant after the beads and attached peptide are pelleted by centrifugation.

Peptides are then released from the beads by the addition of 100μL of 25mM of

imidazole. Samples are then analyzed by standard methods of SDS-PAGE on a 15%

5   polyacrylimide gel. The unmodified PSMGFR peptide runs with an apparent MW of 9

kd, while the modified peptide runs at an apparent MW of 11 kd. A shift to the MW

characteristic of the unmodified peptide, after incubation with lysate and drug candidate,

indicates that the drug under study acts by inhibiting a modifying enzyme.

10   Example 6: Modulation of Inhibitory Effect of Etomoxir on Cell Proliferation

Etomoxir, identified as a composition useful in treatment of MUC1-dependant

tumors in this invention, was shown to be specific for MGFR by modulating its effect on

cell proliferation via competetive inhibition of the MGFR/drug interaction by adding

excess PSMGFR in cell growth media.

15   The following experiment was performed in triplicate. T47D cells were grown to

approximately 30% confluency. Etomoxir (approx. 100 micromolar) was added and cell

proliferation was observed to be arrested. Then, a synthetic peptide (PSMGFR) was

added to the cell growth media under normal cell growth conditions. Addition of

PSMGFR caused increased cell proliferation, due to consumption of Etomoxir by

20   PSMGFR (curve A of Fig. 18). As a control, cells were exposed to Etomoxir and a

control peptide (RGD) of approximately the same molecular weight as PSMGFR and

cell proliferation (curve B of Fig. 18) did not increase to the extent that occurred when

PSMGFR was added.

25   Example 7: Evidence for enzyme modification of the MGFR portion of the MUC1
     receptor

The following experiment was performed to investigate the possibility that the

portion of the MUC1 receptor that remains attached to the cell surface after receptor

cleavage (MGFR) is enzyme-modified.   Synthetic, His-PSMGFR peptides, (SEQ ID

30   NO: 2) were loaded onto NTA-Ni beads and either incubated with T47D cell lysates or

as a negative control, incubated with cell growth media. Incubation was for 1 hour, on

ice with intermittent mixing. As a second negative control, an irrelevant peptide, RGD

(SEQ ID NO: 12) was attached to NTA-Ni beads and treated identically. Both sets of

beads were washed 2X with phosphate buffer pH 7.4. Bound protein species were eluted by addition of 250mM imidazole. Lanes were loaded as follows: (from right to left) (10) pre-stained protein ladder (Gibco); (9) bead immobilized PFMGFR peptide which was not incubated with cell lysates; (8-6) eluates of bead immobilized PFMGFR peptide

5   which was incubated with T47D cell lysates; (5) eluates of bead immobilized negative control peptide which was not incubated with T47D cell lysate; (4-2) eluates of the negative control peptide was incubated with the lysate. Referring now to Fig. 19, comparing lanes 6-8 and 9 (control), it can be seen that after incubation with the lysates (6-8) the MUC1 PSMGFR peptide runs at a higher molecular weight than when it was

10   incubated with cell growth media (9). Comparing lanes 5 and 2-4, there is no change in the apparent molecular weight of the control peptide after incubation with the cell lysate. These results are consistent with the idea that a ligand in the lysate of T47D cells is modifying the PSMGFR peptide portion of the MUC1 receptor.

These results are also consistent with the idea that the ligand is an enzyme that

15   covalently couples two adjacent MGFR portions of the MUC1 receptor, causing dimerization of the receptors and initiates a cell proliferation signaling cascade. According to this mechanism, drugs of the invention interrupt cell proliferation by inhibiting the action of this enzyme.

20   Example 8: Demonstration that the MGFR Peptide is Enzymatically Modified Before its Natural Ligands Recognize It

To test the hypothesis that the MGFR portion of the MUC1 receptor is enzymatically modified prior to binding to its ligand(s), the drug screening assay described in Example 5a was performed in the presence or absence of an enzyme

25   inhibitor, PMSF (phenylmethylsulfonyl fluoride; Sigma chemical Co. St. Louis Missouri, USA). T47D cells, which are breast tumor cells that aberrantly express MUC1, were grown to 70% confluency, treated with trypsin to detach from the flask, and pelleted by centrifugation. A lysate was prepared as follows from the pellets of two T75 flasks. Cell pellets were resuspended in 300 µL phosphate buffer (10mM sodium phosphate, 100mM

30   NaCl, pH 7.4). The lysate was divided into two parts. One aliquot of lysate was used as is. To the second aliquot was added 9µL of a 100 mM stock solution to achieve a final concentration 3 mM. The lysates were frozen and thawed four times with 15 seconds of vigorous mixing after each thawing. Lysates were then pelleted by centrifugation and the

supernatant collected. Lysates were diluted by adding 6.2 ml of phosphate buffer. The drug screening assay was then performed as described in Example 5a, with the exception that no drugs were added. Rather the ability of the ligand(s) in the lysate to bind to the MGFR in a multimeric way, which would result in the solution color to change to blue,

5   was tested. As can be seen in Figure 20, solutions containing PMSF did not change color and remained pink. Wells A 1&2 turned from pink to blue within an hour and are the positive control wells, which contain the His-PSMGFR peptide immobilized on gold colloids and lysates/supernatants from T47D cells. Wells A 3&4 contain the same components as wells A 1&2, with the exception that the lysate/supernatant mixture was

10   first treated with the enzyme inhibitor PMSF; wells containing PMSF do not undergo the solution color change and remain pink. Wells B 1&2 are negative control wells that contain the colloid-immobilized His-PSMGFR peptide but are incubated with buffer rather than lysates, and remain pink. Wells C 1&2 are also negative control wells in which the peptide immobilized on the colloids is an irrelevant peptide (RGD) that is

15   incubated with the lysate, and they remain pink as well.

Prophetic Example Involving Screening for Drugs That Affect MUC 1 Cleavage State

The release of the MUC 1 IBR can be correlated to the progression of cancer. The following is a description of a whole cell assay that identifies drug candidates that

20   affect cleavage state of these receptors. The screen also identifies drug candidates that directly or indirectly modulate any step, including but not limited to enzyme cleavage, receptor production, expression, stability, transport or secretion, that ultimately results in a reduction of the self-aggregating portion of the receptor being shed and released from the cell.

25   Tumor derived cells expressing a cell surface receptor of the type described above, are cultured and treated with a drug candidate. Following some incubation period, a peptide aggregation assay is performed on the solution surrounding the cell. Colloids bearing a binding peptide e.g. an antibody against a constant region of the receptor, remote from the enzyme cleavage site (amino acid 425-479 for MUC1;

30   numbers refer to Andrew Spicer et al., J. Biol. Chem Vol 266 No. 23, 1991 pgs. 15099-15109; these amino acid numbers correspond to numbers 985-1039 of Genbank accession number P15941; PID G547937), are added to the solution. If the shed portion of the receptor contains the self-aggregating portion, the receptors in solution will

aggregate and cause the attached colloids to aggregate, causing a visibly detectable change in the solution, for example: color change or the formation of visible aggregates. An inhibition of this visible change indicates an agent that is effective for treating the disease state.

5   The list of sequences in Table 1 is representative of sequence fragments that are found within the overall sequence of the full-length peptide. Any set of at least 10 contiguous amino acids within any of the sequence fragments of Table 1 may be sufficient to identify the cognate binding motif. The list of sequences of Table 1 is meant to embrace each single sequence and when mentioning fragment size, it is

10   intended that a range embrace the smallest fragment mentioned to the full-length of the sequence (less one amino acid so that it is a fragment), each and every fragment length intended as if specifically enumerated. Thus, if a fragment could be between 10 and 15 in length, it is explicitly meant to mean 10, 11, 12, 13, 14, or 15 in length.

With reference to Table 1, the receptor can be cleaved at a number of different

15   sites to generate peptide fragments with alternative beginnings and endings. For these fragments of Table 1 any stretch of 8 to 10 contiguous amino acids, either upstream or downstream, may be enough to identify the particular fragment that is the binding entity referred to herein.

In addition to the diagnostics and screening assays of the invention, the invention

20   relates to therapeutic methods for the treatment and prevention of cancer and related products. For instance, in one aspect the invention relates to a method for treating a subject having a cancer or at risk of developing cancer by administering to the subject an agent that reduces cleavage of a cell surface receptor IBR from a cell surface receptor.

25   Those skilled in the art would readily appreciate that all parameters listed herein are meant to be exemplary and that actual parameters will depend upon the specific application for which the methods and apparatus of the present invention are used. It is, therefore, to be understood that the foregoing embodiments are presented by way of example only and that, within the scope of the appended claims and equivalents thereto,

30   the invention may be practiced otherwise than as specifically described. Specifically, those of ordinary skill in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention

described herein. Such equivalents are intended to be encompassed by the following claims.

Several methods are disclosed herein of administering a subject with a compound for prevention or treatment of a particular condition. It is to be understood that in each such aspect of the invention, the invention specifically includes, also, the compound for use in the treatment or prevention of that particular condition, as well as use of the compound for the manufacture of a medicament for the treatment or prevention of that particular condition.

In the claims, all transitional phrases such as "comprising", "including", "carrying", "having", "containing", "involving", and the like are to be understood to be open-ended, i.e. to mean including but not limited to. Only the transitional phrases "consisting of" and "consisting essentially of", respectively, shall be closed or semi-closed transitional phrases.

In the claims, amino acid sequence numbers are as listed in Andrew Spicer *et al.*, J. Biol. Chem Vol 266 No. 23, 1991 pgs. 15099-15109.

We Claim:

CLAIMS

1.　　A kit comprising:

　　　　a first article having a surface;

　　　　a peptide sequence immobilized relative to or adapted to be immobilized relative to the surface, the peptide sequence including a portion of a cell surface receptor that interacts with an activating ligand such as a growth factor to promote cell proliferation, the portion including enough of the cell surface receptor to interact with the activating ligand and the portion free of interchain binding region to the extent necessary to prevent spontaneous binding between portions; and

　　　　a candidate drug for affecting the ability of the peptide sequence to bind to other identical peptide sequences in the presence of the activating ligand.

2.　　A kit as in claim 1, further comprising a second article having a surface and the peptide sequence immobilized relative to or adapted to be immobilized relative to the surface of the second article.

3.　　A kit as in claim 1, wherein the peptide sequence is MGFR.

4.　　A method comprising:

　　　　providing a peptide including a portion of a cell surface receptor that interacts with an activating ligand such as a growth factor to promote cell proliferation, the portion including enough of the cell surface receptor to interact with the activating ligand and the portion free of interchain binding region to the extent necessary to prevent spontaneous binding between portions;

　　　　exposing the peptide to a candidate drug for affecting the ability of the activating ligand to interact with the peptide, and to the activating ligand; and

　　　　determining the ability of the candidate drug to prevent interaction of the activating ligand with the peptide.

5.　　A method as in claim 4, comprising determining the ability of the candidate drug to prevent interaction of the peptide with other proteins or peptides.

6.   A method as in claim 4, comprising providing a first article having a surface and a plurality of the peptides immobilized relative to or adapted to be immobilized relative to the surface;

exposing the peptides and the surface of the first article to the candidate drug and at least one activating ligand; and

determining the ability of the candidate drug to prevent interaction of the activating ligand with the peptide.

7.   A method as in claim 4, comprising providing a first article having a surface, a second article having a surface, and a plurality of the peptides immobilized relative to or adapted to be immobilized relative to the surfaces of the first and second articles;

exposing the peptides and the surfaces of the first and second articles to the candidate drug and at least one activating ligand; and

determining immobilization of the first and second articles relative to each other.

8.   A method as in claim 4, wherein the step of exposing the peptides to the candidate drug and at least one activating ligand comprises exposing the peptide and the candidate drug to one or both of cell lysate and cell supernatant containing the activating ligand.

9.   A method as in claim 4, wherein the peptide sequence is PSMGFR.

10.   A method of treating a subject to reduce the risk of or progression of cancer comprising:

administering to a subject who is known to be at risk for cancer or is diagnosed with cancer an agent for inhibiting interaction of an activating ligand with a portion of a cell surface receptor that interacts with the activating ligand to promote cell proliferation.

11.   A method as in claim 10, comprising administering to the subject an agent for inhibiting inductive multimerization of the portion of the cell surface receptor that interacts with the activating ligand to promote cell proliferation.

12.   A method as in claim 10, wherein the cell surface receptor is MUC1.

13.   A method as in claim 10, wherein the portion of the cell surface receptor is MGFR.

5   14.   A method as in claim 10, wherein the portion of the cell surface receptor contains a significant part of the PSMGFR sequence.

15.   A method as in claim 10, comprising administering to the subject an agent for inhibiting dimerization of the portion of the cell surface receptor that interacts with the
10   activating ligand to promote cell proliferation.

16.   The method of claim 10, wherein the cancers is selected from the group consisting of: breast, prostate, lung ovarian, colorectal, and brain cancer.

15   17.   A method as in claim 10, wherein the activating ligand is a multimer.

18.   The method of claim 10, wherein the activating ligand is a protein with a molecular weight of about 17kD.

20   19.   The method of claim 10, wherein the activating ligand is a protein with a molecular weight of about 23kD.

20.   The method of claim 10, wherein the activating ligand is a protein with a molecular weight of about 35kD.

25
21.   The method of claim 10, wherein the activating ligand contains sequences derived from the protein 14-3-3.

22.   The method of claim 10, wherein the activating ligand contains sequences
30   derived from cathepsin D.

23.   The method of claim 10, wherein the activating ligand contains sequences derived from NM23.

24.   The method of claim 10, wherein the activating ligand contains sequences derived from human annexin V.

5   25.   The method of claim 10, wherein the activating ligand contains sequences derived from beta-lipotropin.

26.   The method of claim 10, wherein the activating ligand is a cleavage product of proopiomelanocortin.

10

27.   The method of claim 10, wherein the portion of the cell surface comprises at least 12 contiguous amino acids from the sequence GTINVHDVETQFNQYKTEAASPYNLTISDVSVSDVPFPFSAQSGA.

15   28.   The method of claim 10, wherein the portion of the cell surface receptor that remains attached to the cell surface after shedding of the cell surface receptor interchain binding region comprises at least 12 contiguous amino acids from the peptide sequence GTINVHDVETQFNQYKTEAASPYNLTISDVSVS.

20   29.   The method of claim 10, wherein the agent is selected for use in the method by determining its ability to bind to a significant portion of the peptide, GTINVHDVETQFNQYKTEAASPYNLTISDVSVSDVPFPFSAQSGA.

30.   The method of claim 10, wherein the agent is selected for use in the method by
25   determining its ability to bind to a significant portion of the peptide sequence GTINVHDVETQFNQYKTEAASPYNLTISDVSVS.

31.   A method of treating a subject to reduce the risk or of progression of cancer comprising:
30   administering to a subject who is known to be at risk of cancer or is diagnosed with cancer, an agent for preventative clustering of portions of cell surface receptors that interact with an activating ligand such as a growth factor to promote cell proliferation.

32.   The method of claim 31, wherein the cell surface receptor is MUC1.

33.   A method as in claim 31, comprising contacting the portions of the cell surface
receptor with a molecule that can bind to multiple portions thereby clustering a plurality
of the portions.

34.   A method as in claim 31, wherein the portion is MGFR.

35.   A method as in claim 31, wherein the portion contains a significant amount of the
PFMGFR sequence.

36.   The method of claim 31, wherein the cancer is selected from the group consisting
of: breast, prostate, lung ovarian, colorectal, and brain cancer.

37.   The method of claim 31, wherein the portion of the cell surface receptor
comprises at least 12 contiguous amino acids from the peptide sequence
GTINVHDVETQFNQYKTEAASPYNLTISDVSVSDVPFPFSAQSGA.

38.   The method of claim 31, wherein the portion of the cell surface receptor
comprises at least 12 contiguous amino acids from the peptide sequence
GTINVHDVETQFNQYKTEAASPYNLTISDVSVS.

39.   The method of claim 31, wherein the specific binding portion of the agent is
selected for use in the method by determining its ability to bind to a significant portion of
the peptide, GTINVHDVETQFNQYKTEAASPYNLTISDVSVSDVPFPFSAQSGA.

40.   The method of claim 31, wherein the specific binding portion of the agent is
selected for use in the method by determining its ability to bind to a significant portion of
the peptide, GTINVHDVETQFNQYKTEAASPYNLTISDVSVS.

41.   A kit comprising:

a species able to become immobilized relative to a shed cell surface receptor interchain binding region; and

a signaling entity immobilized relative to or adapted to be immobilized relative to the species.

42.   A kit as in claim 41, wherein the species binds to a portion of a shed MUC1 receptor that is connected to the interchain binding region.

43.   A kit as in claim 41, wherein the cell surface receptor is MUC1.

44.   The kit as in claim 41, wherein the signaling entity is a colloid particle.

45.   The kit as in claim 41, wherein the signaling entity is not a colloid particle.

46.   The colloid particle as in claim 41, further comprising a colloid particle, wherein the signaling entity is attached to the colloid particle.

47.   A composition comprising:

at least a portion of a shed cell surface receptor interchain binding region; and

a signaling entity immobilized relative to or adapted to be immobilized relative to the portion.

48.   A kit comprising:

a species able to bind to a portion of a cell surface receptor that remains attached to the cell surface after shedding of a cell surface receptor interchain binding region; and

a signaling entity immobilized relative to or adapted to be immobilized relative to the species.

49.   A kit as in claim 48, wherein the cell surface receptor is MUC1.

50.   The kit as in claim 48, wherein the signaling entity is a colloid particle.

51.   The kit as in claim 48, wherein the signaling entity is not a colloid particle.

52.   The kit particle as in claim 48, further comprising a colloid particle, wherein the signaling entity is attached to the colloid particle.

53.   The kit as in claim 48, wherein the species able to bind to a portion of a cell surface receptor that remains attached to the cell surface after shedding of a cell surface receptor interchain binding region is a protein of about 17kD.

54.   The kit as in claim 49, wherein the species able to bind to a portion of a cell surface receptor that remains attached to the cell surface after shedding of a cell surface receptor interchain binding region is a protein of about 23kD.

55.   The kit as in claim 49, wherein the species able to bind to a portion of a cell surface receptor that remains attached to the cell surface after shedding of a cell surface receptor interchain binding region is a protein of about 35Kd.

56.   The kit as in claim 49, wherein the species able to bind to a portion of a cell surface receptor that remains attached to the cell surface after shedding of a cell surface receptor interchain binding region contains sequences derived from the protein 14-3-3.

57.   The kit as in claim 49, wherein the portion comprises 14-3-3.

58.   The kit as in claim 49, wherein the portion comprises cathepsin D.

59.   The kit as in claim 49, wherein the portion comprises NM23.

60.   The kit as in claim 49, wherein the portion comprises Human annexin V.

61.   The kit as in claim 49, wherein the species able to bind to a portion of a cell surface receptor that remains attached to the cell surface after shedding of a cell surface receptor interchain binding region contains at least one sequence derived from beta-lipotropin.

62. The kit as in claim 49, wherein the species able to bind to a portion of a cell surface receptor that remains attached to the cell surface after shedding of a cell surface receptor interchain binding region is a cleavage product of proopiomelanocortin.

63. A kit comprising:

a species able to bind to a portion of a cell surface receptor that includes the interchain binding region; and

a signaling entity immobilized relative to or adapted to be immobilized relative to the species.

64. A kit as in claim 63, wherein the cell surface receptor is MUC1.

65. The kit as in claim 63, wherein the signaling entity is a colloid particle.

66. The kit as in claim 63, wherein the signaling entity is not a colloid particle.

67. The colloid particle as in claim 63, further comprising a colloid particle, wherein the signaling entity is attached to the colloid particle.

68. A peptide species comprising:

at least a fragment of a sequence that corresponds to that portion of a cell surface receptor that interacts with an activating ligand such as a growth factor to promote cell proliferation, the portion being detached from any cell; and

an affinity tag.

69. A peptide species as in claim 68, wherein the affinity tag is connected to the fragment.

70. A peptide species as in claim 68, wherein the affinity tag defines a portion of a continuous amino acid sequence that includes both the fragment and the affinity tag.

71. The species of claim 68, wherein the affinity tag is a polyamino acid tag.

72.   The species of claim 68, wherein the affinity tag is a polyhistidine tag.

73.   The species of claim 68, wherein the affinity tag is a GST tag.

5

74.   The species of claim 68, wherein the affinity tag is biotin.

75.   The species of claim 68, wherein the affinity tag is Thioredoxin.

10   76.   The species of claim 68, wherein the affinity tag is selected to bind to a species
immobilizied with respect to the surface of an article.

77.   The species of claim 68, further comprising an article having a surface, and a
species able to capture the affinity tag immobilizied with respect to the surface.

15

78.   The species of claim 68, wherein the article is a particle.

79.   The species of claim 68, wherein the affinity tag is fastened to the C-terminus of
the portion of the receptor.

20

80.   The species of claim 68, wherein the cell surface receptor is MUC1.

81.   The species of claim 68, wherein the cell surface receptor portion comprises 12 or
more contiguous amino acids in the sequence

25   GTINVHDVETQFNQYKTEAASPYNLTISDVSVSDVPFPSAQSGA (SEQ ID NO: 7).

82.   A peptide species as in claim 68, wherein the fragment comprises at least a
portion of PSMGFR.

30   83.   A peptide species as in claim 68, wherein the fragment comprises PSMGFR.

84.   A peptide species as in claim 68, wherein the fragment comprises at least a
fragment of the sequence that corresponds to that portion of MUC1 that interacts with an

activating ligand such as a growth factor to promote cell proliferation in association with MUC1-dependent tumorigenesis.

85.   A peptide species as in claim 68, wherein the fragment comprises enough of the sequence that corresponds to that portion of MUC1 that remains attached to the cell surface after shedding of the cell surface receptor interchain binding region in association with MUC1-dependent tumorigenesis such that a biomolecule that interacts with that portion of MUC1 that remains attached to the cell surface after shedding of the cell surface receptor interchain binding region in association with MUC1-dependent tumorigenesis interacts with the fragment.

86.   A kit comprising:
        a particle; and
        at least a fragment of the sequence that corresponds to that portion of a cell surface receptor that interacts with an activating ligand such as a growth factor to promote cell proliferation, the fragment being detached from any cell, fastened to or adapted to be fastened to the particle.

87.   The kit of claim 86, wherein the cell surface receptor is MUC1.

88.   A kit comprising:
        an article having a surface; and
        a biomolecule that binds to a portion of a cell surface receptor that interacts with an activating ligand such as a growth factor to promote cell proliferation, the biomolecule being fastened to or adapted to be fastened to the surface of the article.

89.   The kit of claim 88, wherein the article comprises a particle.

90.   The kit of claim 88, wherein the cell surface receptor is MUC1.

91.   The kit of claim 88, further comprising:
        a second particle; and

a portion of a cell surface receptor that remains attached to the cell surface after shedding of the cell surface receptor interchain binding region, the portion being detached from any cell, fastened to or adapted to be fastened to the second particle.

5   92.   The kit as in claim 88, wherein the biomolecule that binds to a portion of a cell surface receptor that remains attached to the cell surface after shedding of a cell surface receptor interchain binding region is a protein of about 17kD.

93.   The kit as in claim 88, wherein the biomolecule that binds to a portion of a cell
10   surface receptor that remains attached to the cell surface after shedding of a cell surface receptor interchain binding region is a protein of about 23kD.

94.   The kit as in claim 88, wherein the biomolecule that binds to a portion of a cell surface receptor that remains attached to the cell surface after shedding of a cell surface
15   receptor interchain binding region is a protein of about 35kD.

95.   The kit as in claim 88, wherein the biomolecule that binds to a portion of a cell surface receptor that remains attached to the cell surface after shedding of a cell surface receptor interchain binding region contains sequences derived from the protein 14-3-3.

20
96.   The kit as in claim 88, wherein the biomolecule that binds to a portion of a cell surface receptor that remains attached to the cell surface after shedding of a cell surface receptor interchain binding region contains sequences derived from Cathepsin D.

25   97.   The kit as in claim 88, wherein the biomolecule that binds to a portion of a cell surface receptor that remains attached to the cell surface after shedding of a cell surface receptor interchain binding region contains sequences derived from NM23.

98.   The kit as in claim 88, wherein the biomolecule that binds to a portion of a cell
30   surface receptor that remains attached to the cell surface after shedding of a cell surface receptor interchain binding region contains sequences derived from human annexin V.

99.   The kit as in claim 88, wherein the biomolecule that binds to a portion of a cell surface receptor that remains attached to the cell surface after shedding of a cell surface receptor interchain binding region contains at least one sequence derived from beta-lipotropin.

100.   The kit as in claim 88, wherein the biomolecule that binds to a portion of a cell surface receptor that remains attached to the cell surface after shedding of a cell surface receptor interchain binding region is a cleavage product of proopiomelanocortin.

101.   The kit as in claim 88, wherein the biomolecule that binds to a portion of a cell surface receptor that remains attached to the cell surface after shedding of a cell surface receptor interchain binding region is selected from the group which includes calcimycin, fusaric acid, L-α-methyl-dopa, and etomoxir.

102.   The kit as in claim 88, wherein the biomolecule that binds to a portion of a cell surface receptor that remains attached to the cell surface after shedding of a cell surface receptor interchain binding region comprises calcimycin.

103.   The kit as in claim 88, wherein the biomolecule that binds to a portion of a cell surface receptor that remains attached to the cell surface after shedding of a cell surface receptor interchain binding region comprises fusaric acid.

104.   The kit as in claim 88, wherein the biomolecule that binds to a portion of a cell surface receptor that remains attached to the cell surface after shedding of a cell surface receptor interchain binding region comprises L-α-methyl-dopa.

105.   The kit as in claim 88, wherein the biomolecule that binds to a portion of a cell surface receptor that remains attached to the cell surface after shedding of a cell surface receptor interchain binding region comprises etomoxir.

106.   The composition of claim 88, wherein the biomolecule is derived from a cell line selected from the group consisting of HTB-133, CRL-1504, and CRL-1500.

107.   A method comprising:

exposing a ligand capable of binding with a portion of a cell surface receptor that remains attached to the cell surface after shedding of a cell surface receptor interchain

5   binding region, and an agent capable of blocking said binding, to a candidate drug for disruption of interaction between the ligand and the agent; and

determining disruption of the interaction by the candidate drug.

108.   A method comprising:

10   exposing a portion of a cell surface receptor that remains attached to the cell surface after shedding of a cell surface receptor interchain binding region which is capable of binding with a ligand, and an agent capable of blocking said binding, to a candidate drug for disruption of interaction between the portion and the agent; and

determining disruption of the interaction by the candidate drug.

15

109.   A method comprising:

exposing a synthetic drug, and a biological target of the synthetic drug, to a candidate drug which may interact with the biological target to a degree greater than the interaction between the synthetic drug and the target; and

20   determining disruption of the interaction by the candidate drug.

110.   A method as in claim 109, wherein the synthetic drug is a derivative of fusaric acid.

25   111.   A method as in claim 109, wherein the synthetic drug is a derivative of L-α-methyl-dopa.

112.   A method as in claim 109, wherein the synthetic drug is a derivative of etomoxir.

30   113.   A method for treating a subject having a cancer characterized by the aberrant expression of MUC1, comprising:

administering to the subject fusaric acid in an amount effective to reduce tumor growth.

114.   A method as in claim 113, wherein the subject is otherwise free of symptoms calling for treatment with calcimycin.

115.   A method as in claim 113, wherein the method comprises administering to the subject fusaric acid in an amount effective to block the interaction of a natural ligand and the portion of the MUC1 receptor that remains attached to the cell surface after shedding of the cell surface receptor interchain binding region.

116.   A method as in claim 113, wherein the method comprises administering to the subject fusaric acid in an amount effective to reduce shedding of the interchain binding region of the MUC1 receptor.

117.   The method of 113, wherein the levels of shed interchain binding region are reduced relative to a level measured in a past sample.

118.   The method of 113, wherein the levels of shed interchain binding region are reduced relative to a control sample.

119.   A method for treating a subject having a cancer characterized by the aberrant expression of MUC1, comprising:
        administering to the subject etomoxir in an amount effective to reduce tumor growth.

120.   A method as in claim 119, wherein the subject is otherwise free of symptoms calling for treatment with etomoxir.

121.   A method as in claim 119, wherein the method comprises administering to the subject etomoxir in an amount effective to block the interaction of a natural ligand and the portion of the MUC1 receptor that remains attached to the cell surface after shedding of the cell surface receptor interchain binding region.

122.   A method as in claim 119, wherein the method comprises administering to the subject etomoxir in an amount effective to reduce shedding of the interchain binding region of the MUC1 receptor.

5

123.   The method of 119, wherein the levels of shed interchain binding region are reduced relative to a level measured in a past sample.

124.   The method of 119, wherein the levels of shed interchain binding region are reduced relative to a control sample.

10

125.   A method for treating a subject having a cancer characterized by the aberrant expression of MUC1, comprising:

administering to the subject L-α-methyl-dopa in an amount effective to reduce tumor growth.

15

126.   A method as in claim 125, wherein the subject is otherwise free of symptoms calling for treatment with L-α-methyl-dopa.

127.   A method as in claim 125, wherein the method comprises administering to the subject L-α-methyl-dopa in an amount effective to block the interaction of a natural ligand and the portion of the MUC1 receptor that remains attached to the cell surface after shedding of the cell surface receptor interchain binding region.

20

128.   A method as in claim 125, wherein the method comprises administering to the subject L-α-methyl-dopa in an amount effective to reduce shedding of the interchain binding region of the MUC1 receptor.

25

129.   The method of 125, wherein the levels of shed interchain binding region are reduced relative to a level measured in a past sample.

30

130.   The method of 125, wherein the levels of shed interchain binding region are reduced relative to a control sample.

131.   A method for treating a subject having a cancer characterized by the aberrant

5   expression of MUC1, comprising:

administering to the subject calcimycin in an amount effective to reduce tumor growth.

132.   A method as in claim 131, wherein the subject is otherwise free of symptoms

10   calling for treatment with calcimycin.

133.   A method as in claim 131, wherein the method comprises administering to the subject calcimycin in an amount effective to block the interaction of a natural ligand and the portion of the MUC1 receptor that remains attached to the cell surface after shedding

15   of the cell surface receptor interchain binding region.

134.   A method as in claim 131, wherein the method comprises administering to the subject calcimycin in an amount effective to reduce shedding of the interchain binding region of the MUC1 receptor.

20

135.   The method of 134, wherein the levels of shed interchain binding region are reduced relative to a level measured in a past sample.

136.   The method of 134, wherein the levels of shed interchain binding region are

25   reduced relative to a control sample.

137.   A method for treating a subject having a cancer characterized by the aberrant expression of MUC1, comprising:

administering to the subject butylindazole in an amount effective to reduce tumor

30   growth.

138.   A method as in claim 137, wherein the subject is otherwise free of symptoms calling for treatment with butylindazole.

139.   A method as in claim 137, wherein the method comprises administering to the subject butylindazole in an amount effective to block the interaction of a natural ligand and the portion of the MUC1 receptor that remains attached to the cell surface after
5   shedding of the cell surface receptor interchain binding region.

140.   A method as in claim 137, wherein the method comprises administering to the subject butylindazole in an amount effective to reduce shedding of the interchain binding region of the MUC1 receptor.
10

141.   The method of 137, wherein the levels of shed interchain binding region are reduced relative to a level measured in a past sample.

142.   The method of 137, wherein the levels of shed interchain binding region are
15   reduced relative to a control sample.

143.   A method for treating a subject having a cancer characterized by the aberrant expression of MUC1, comprising:
        administering to the subject NS1619 in an amount effective to reduce tumor
20   growth.

144.   A method as in claim 143, wherein the subject is otherwise free of symptoms calling for treatment with NS1619.

25   145.   A method as in claim 143, wherein the method comprises administering to the subject NS1619 in an amount effective to block the interaction of a natural ligand and the portion of the MUC1 receptor that remains attached to the cell surface after shedding of the cell surface receptor interchain binding region.

30   146.   A method as in claim 143, wherein the method comprises administering to the subject NS1619 in an amount effective to reduce shedding of the interchain binding region of the MUC1 receptor.

147. The method of 143, wherein the levels of shed interchain binding region are reduced relative to a level measured in a past sample.

148. The method of 143, wherein the levels of shed interchain binding region are reduced relative to a control sample.

149. A method comprising:

exposing a composition selected among calcimycin, butylindazone, NS1619, fusaric acid, L-α-methyl-dopa, and etomoxir, and a biomolecule that binds to a portion of a cell surface receptor that remains attached to the cell surface after shedding of a cell surface receptor interchain binding region, to a candidate drug which may interfere with interaction between the composition and the biomolecule; and

determining disruption of the interaction by the candidate drug.

150. A method of treating a subject having cancer or at risk for developing cancer comprising:

administering to the subject an agent that reduces cleavage of a cell surface receptor.

151. A method of treating a subject having cancer or at risk for developing cancer comprising:

administering to the subject an agent that reduces cleavage of a cell surface receptor interchain binding region from the cell surface.

152. The method of claim 150, wherein the cell surface receptor is MUC1.

153. The method of claim 151, wherein the interchain binding region comprises a contiguous amino acid sequence of at least 12 amino acids from the sequence GFLGLSNIKFRPGSVVVQLTLAFRE.

154. The method of claim 150, wherein the interchain binding region comprises a contiguous amino acid sequence of about 12 to 18 amino acids, within the region of the

human MUC1 receptor amino acids 507 through 549 (refers to Spicer et al sequence –

corresponds to amino acids 1067 through 1100 of Genbank accession # PI5941, PID

G547937).

5   155.   The method of claim 150, wherein the interchain binding region comprises a

contiguous amino acid sequence of about 12 to 18 amino acids, within the region of the

human MUC1 receptor amino acids 525 through 549 (refers to Spicer et al sequence –

corresponds to amino acids 1085 through 1109 of Genbank accession # PI5941, PID

G547937).

10

156.   The method of claim 150, wherein the cancer is selected from the group

consisting of: breast, prostate, lung ovarian, colorectal, and brain cancer.

157.   The method of claim 150, wherein the cancer is characterized by the aberrant

15   expression of the MUC1 receptor.

158.   A method comprising:

        determining an amount of cleavage of a cell surface receptor interchain binding

region from a cell surface; and

20   evaluating indication of cancer or potential for cancer based upon the determining

step.

159.   A method as in claim 158, wherein the cell surface receptor is MUC1.

25   160.   A method as in claim 158, comprising diagnosing cancer in a subject by

determining an amount of shed cell surface receptor interchain binding region in a

subject sample; and

        evaluating indication of cancer or potential for cancer based upon the determining

step.

30

161.   A method as in claim 158, wherein the evaluating step comprises correlating the

amount in a sample to an amount in a control as an indication of cancer or potential for

cancer.

162. A method as in claim 158, comprising:

determining an amount of cell surface receptor interchain binding region at the surface of a cell from a subject; and

5   evaluating indication of cancer or potential for cancer based upon the determining step.

163. The method of claim 158, wherein the interchain binding region comprises a contiguous amino acid sequence of at least 12 amino acids from the sequence

10   GFLGLSNIKFRPGSVVVQLTLAFRE.

164. The method of claim 158, wherein the interchain binding region comprises a contiguous amino acid sequence of about 12 to 18 amino acids, within the region of the human MUC1 receptor amino acids 507 through 549 (refers to Spicer et al sequence –

15   corresponds to amino acids 1067 through 1100 of Genbank accession # PI5941, PID G547937).

165. The method of claim 158, wherein the interchain binding region comprises a contiguous amino acid sequence of about 12 to 18 amino acids, within the region of the

20   human MUC1 receptor amino acids 525 through 549 (refers to Spicer et al sequence – corresponds to amino acids 1085 through 1109 of Genbank accession # PI5941, PID G547937).

166. The method of claim 160, wherein the sample is a fluid sample.

25

167. The method of claim 160, wherein the sample is blood.

168. The method of claim 160, wherein the sample is a tissue sample.

30   169. The method of claim 160, wherein the sample is a proliferating cell line derived from a subject's cells.

170. The method of claim 158, wherein the cancer is characterized by aberrant expression of MUC1.

171. The method of claim 158, wherein the amount of interchain binding region is determined by a method selected from the group consisting of MALDI, western blotting, PCR, LCR, rtPCR, cycling probe technology, gel electrophoresis, or antibody-based assay, magnetic cell sorting, flourescence activated cell sorting, bead-based assays or an ELISA assay.

172. The method of claim 158, wherein the amount of interchain binding region is determined by an aggregation assay.

173. The method of claim 158, wherein the amount of interchain binding region is determined by a colloid-based method such as colloid-colloid or colloid-bead assay.

174. The method of claim 158, wherein the sample is selected from the group consisting of: a needle biopsy, a tissue specimen, a tissue surface in an intraoperative procedure, and a tissue surface or cellular solution in a minimally invasive procedure such as a laparoscopy.

175. A method comprising:
determining a site of cleavage of a cell surface receptor in a sample from a subject; and
evaluating an indication of cancer or potential for cancer based upon the determining step.

176. The method of claim 175, wherein the cell surface receptor is MUC1.

177. The method of claim 175, wherein the sample is selected from the group consisting of: a needle biopsy, a tissue specimen, a tissue surface in an intraoperative procedure, and a tissue surface or cellular solution in a minimally invasive procedure such as a laparoscopy.

178.   The method of claim 175, wherein the sample is a fluid sample.

179.   The method of claim 175, wherein the sample is blood.

5   180.   The method of claim 175, wherein the sample is a tissue sample.

181.   The method of claim 175, wherein the cancer is selected from the group
consisting of: breast, prostate, lung, ovarian, colorectal, and brain cancer.

10   182.   A method as in claim 175, wherein the cancer is characterized by the aberrant
expression of MUC1.

183.   The method of claim 175, wherein the site of cleavage is determined by a
method selected from the group consisting of MALDI, western blotting, PCR, LCR,
15   rtPCR, cycling probe technology, gel electrophoresis, or antibody-based assay, magnetic
cell sorting, flourescence activated cell sorting, bead-based assays or an ELISA assay.

184.   The method of claim 175, wherein the amount of interchain binding region is
determined by a colloid-based method such as colloid-colloid or colloid-bead assay.

20
185.   A method of determining a cleavage site of a cell surface comprising:
        contacting a cell with an agent that binds specifically to one potential cell surface
receptor cleavage site and another agent that binds specifically to another potential cell
surface receptor cleavage site; and
25   comparing the ratio of binding of the two agents to the cell surface.

186.   The method of claim 185, wherein the surface cell receptor is MUC1.

187.   A method of diagnosing a physiological state indicative of cancer or potential for
30   cancer, comprising determining a specific cleavage state of MUC 1 distinguishable from
a different cleavage state of MUC1.

188.   A method comprising:

determining a first amount of cleavage of a cell surface receptor interchain binding region from a cell surface of a sample from a subject;
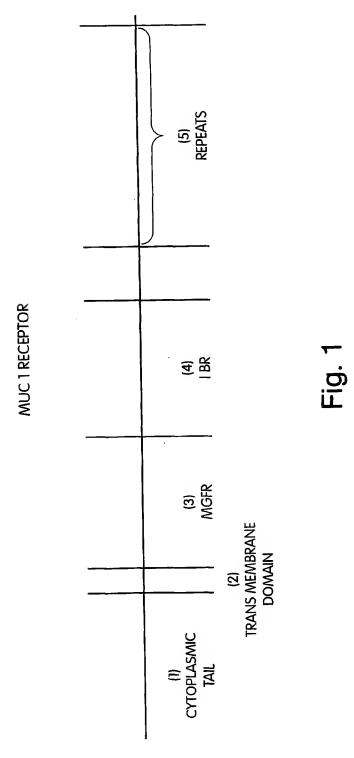
determining a second amount of cleavage of a cell surface receptor interchain

5   binding region from a cell surface of a sample from the subject;

comparing the first amount to the second amount.

189.   A method as in claim 188, comprising comparing the first amount to the second amount as an indication of progression of and/or effectiveness of treatment for cancer.

10

190.   A method as in claim 188, comprising comparing the first amount to the second amount as an indication for administration of an agent for prevention of cancer.

191.   A method as in claim 188, wherein the subject is undergoing treatment for

15   cancer, the method comprising

comparing the first amount to the second amount as an indication of effectiveness of the treatment.

192.   A method as in claim 188, wherein the cell surface receptor is MUC1.

20

193.   The method of claim 192, wherein the interchain binding region comprises a contiguous amino acid sequence of at least 12 amino acids from the sequence GFLGLSNIKFRPGSVVVQLTLAFRE.

25   194.   The method of claim 192, wherein the interchain binding region comprises a contiguous amino acid sequence of about 12 to 18 amino acids, within the region of the human MUC1 receptor amino acids 507 through 549 (refers to Spicer et al sequence – corresponds to amino acids 1067 through 1100 of Genbank accession # PI5941, PID G547937).

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195.   The method of claim 188, wherein the interchain binding region comprises a contiguous amino acid sequence of about 12 to 18 amino acids, within the region of the human MUC1 receptor amino acids 525 through 549 (refers to Spicer et al sequence –

corresponds to amino acids 1085 through 1109 of Genbank accession # PI5941, PID G547937).

196.   The method of claim 188, wherein the sample is a fluid sample.

197.   The method of claim 188, wherein the sample is blood.

198.   The method of claim 188, wherein the sample is a tissue sample.

199.   The method of claim 188, wherein the cancer is selected from the group consisting of: breast, prostate, lung, ovarian, colorectal, and brain cancer.

200.   The method of claim 188, wherein the sample is a proliferating cell line derived from a patient's cells.

201.   The method of claim 188, wherein the amount of interchain binding region is determined by a method selected from the group consisting of MALDI, western blotting, PCR, LCR, rtPCR, cycling probe technology, gel electrophoresis, or antibody-based assay, magnetic cell sorting, flourescence activated cell sorting, bead-based assays or an ELISA assay.

202.   The method of claim 188, wherein the amount of interchain binding region is determined by an aggregation assay.

203.   The method of claim 188, wherein the amount of interchain binding region is determined by a colloid-based method such as colloid-colloid or colloid-bead assay.

204.   The method of claim 188, wherein the sample is selected from the group consisting of: a needle biopsy, a tissue specimen, a tissue surface in an intraoperative procedure, and a tissue surface or cellular solution in a minimally invasive procedure such as a laparoscopy.

205.   The method of claim 188, wherein the amount of interchain binding region is determined by a colloid-based method such as colloid-colloid or colloid-bead assay.

206.   The method of claim 188, wherein the sample is selected from the group
consisting of: a needle biopsy, a tissue specimen, a tissue surface in an intraoperative procedure, and a tissue surface or cellular solution in a minimally invasive procedure such as a laparoscopy.

207.   A method as in claim 188, comprising determining a first amount of a cell surface receptor interchain binding region at the surface of a cell in a sample from a subject,
        determining a second amount of a cell surface receptor interchain binding region at the surface of a cell in a sample from the subject,
        comparing the first amount to the second amount.

208.   A method as in claim 188, comprising determining a first amount of a shed cell surface receptor interchain binding region in a sample from a subject,
        determining a second amount of a shed cell surface receptor interchain binding region in a sample from the subject,
        comparing the first amount to the second amount.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

FOR A-D WELLS

ALL P ALL P ALL P ALL P ALL P ALL P ALL P ALL P ALL P ALL P ALL P ALL P

E-H WELLS ALL P

P = PINK
B = BLUE
I = INTERMEDIATE

Fig. 8A

Fig. 8B

FOR A-D   ALL I ALL B ALL P ALL I ALL B ALL P ALL I ALL B ALL P ALL I ALL B ALL P
WELLS

P = PINK
B = BLUE
I = INTERMEDIATE

Fig. 8C

Fig. 8D

1 2 3 4 5 6 7 8 9 10

35KD
23KD
17KD

Fig. 9

Fig. 10

Fig. 11

Fig. 12

Fig. 13

Fig. 14

Fig. 15

Fig. 16

Fig. 17

Fig. 18

Fig. 19

PSMGFR COLLOID
+ LYSATE (-PMSF)(BLUE)

PSMGFR COLLOID
+ LYSATE (+PMSF)(PINK)

PSMGFR COLLOID
+ BUFFER (PINK)

RGD COLLOID
+ LYSATE (PINK)

Fig. 20